STUDIES ON THE OSMOREGULATORY PHYSIOLOGY OF A FRESHWATER TELEOST FISH

THESIS SUBMITTED FOR THE AWARD OF THE DEGREE OF

Doctor of Philosophy

IN

ZOOLOGY

BY

FAUZIA ANWAR SHERWANI

DEPARTMENT OF ZOOLOGY
ALIGARH MUSLIM UNIVERSITY
ALIGARH (INDIA)

JUNE, 1997

This is to certify that the thesis entitled " **Studies on the osmoregulatory physiology of a freshwater teleost fish** " which is being submitted by Ms. *Fauzia Anwar Sherwani* embodies *original work done by the candidate* herself. The entire work was carried out under my supervision and I allow her to submit the same in fulfillment of the requirements for the degree of **Doctor of Philosophy** in Zoology of this University.

IQBAL PARWEZ
(Supervisor)

STUDIES ON THE OSMOREGULATORY PHYSIOLOGY OF A FRESHWATER TELEOST FISH

CONTENTS

PREFACE

The internal milieu of the majority of aquatic animals is either hypo- or hyper-osmotic with respect to their external environment. These animals are subjected to various osmotic adjustments of their body fluid and are able to adapt to a particular environment. The euryhalinity among the aquatic animals illustrates their capacity to successfully adapt in an osmotically different medium which is mainly due to the versatility of their osmoregulatory mechanism. The physiology of salt and water balance has always been a fascinating area of investigation, the evidence of which comes from enormous amount of the work relating to ion-induced structural changes in target organs (Zwingelstein *et al.*, 1980), biometric changes (Boeuf and Lasserre, 1979; Alliot, 1982), endocrinological modifications (Foskett *et al.*, 1983; Nichols and Weisbart, 1985; Olsen *et al.*, 1993; Cornell *et al.*, 1994, Marc *et al.*, 1995) and the changes in metabolic processes (Roche *et al.*, 1983; Roche and Pérès, 1983; Roche, *et al.*, 1989; Mayer *et al.*, 1994; Woo and Chung, 1995; Soengas *et al.*, 1995a, b).

In its simplest term, osmoregulation may be defined as regulation of the osmotic concentration of the body fluids. It is the process of maintaining the amounts of water and

specific solutes in body compartments within the narrow limits of tolerance. Since the cellular metabolism which defines life is possible only in a milieu whose ionic and organic solute concentration is relatively consistent, it becomes necessary to elucidate the osmoregulatory strategies and the array of mechanisms that the organisms have evolved to cope with the challenges imposed by the diverse environment. The physiology of regulation of salt and water balance in aquatic animals, living in a highly divergent environment ranging from salt-rich sea water to an extremely salt-deficient fresh water, shows unique adaptive patterns in their respective environments. To cope with the challenges imposed by the osmotic variability of the aquatic environment, fishes have adopted several strategies. For example, in some groups of fishes, the osmotic gradient between internal and the external medium has been virtually eliminated by maintaining electrolyte concentration of the body fluids almost equal to that of sea water, as in hagfishes (Holmes and Donaldson, 1969). In another group, the osmotic gradient between the internal milieu of the fish and the outside environment is virtually discarded by retaining large amounts of nitrogenous compounds such as urea, trimethylamine oxide, as in marine elasmobranchs (Holmes and Donaldson, 1969). In such fishes, the osmotic pressure of

the extra-cellular fluid is equal to or slightly higher than that of the sea water corresponding to 35‰ salinity. Even though the extra-cellular fluid has the same osmotic pressure, it differs form sea water in ionic composition. Therefore, even in these fishes ionic regulation occurs. Such fishes can endure relatively small fluctuations in the osmotic pressure of their environment and their distribution is generally restricted to sea.

In freshwater fishes, isotonicity cannot be maintained since no animal can withstand the low osmotic pressure of fresh water in its cells or blood. Therefore, another strategy adopted by teleost fish which thrive in both fresh water and sea water involves maintenance of internal osmotic pressure almost equivalent to a third of the salinity of sea water. The teleost fishes are, therefore, hypo-osmotic regulators in sea water and hyper-osmotic regulators in fresh water.

Studies on the osmoregulatory physiology of teleosts have shown that the strategy adopted by a freshwater teleost to maintain its osmo-ionic integrity is diagonally opposite to that of its marine counterpart. The classical model proposed by Smith (1932) and Krogh (1939) to explain mechanisms operating in freshwater and marine teleosts is still essentially valid. In freshwater teleosts, water

entering by osmosis has to be continually eliminated with minimal loss of salts. Formation of large quantities of urine is, therefore, a characteristic feature of freshwater teleosts. The ionic loss is made up principally by branchial uptake of salts from the environment against a concentration gradient and to some extent, from the ingested food material. Thus, the kidney and the gills are the principal organs in maintaining homeostasis in freshwater teleosts. In contrast, marine teleosts combat osmotic dehydration by drinking sea water from which, water with monovalent and some divalent ions, is absorbed through the gut, leaving behind most of the divalent ions to be excreted along with the faecal matter. The extra salt load due to influx through the gills and the absorption from the gut is excreted by the kidney in the form of highly concentrated urine as well as through extra-renal pathways, principally the gills. Thus, in marine teleosts, osmo-ionic balance is maintained by the coordination between the gastrointestinal tract and the renal and branchial complex. Further, marine teleosts maintain the regulated tonicity of their body fluids slightly higher than their freshwater counterparts.

Based on the tolerance and the regulatory processes against the changes in external salinity, fish may be categorized in two different types __ the euryhaline fishes

are able to cope with gradual or abrupt transfers from fresh water to sea water and *vice versa*, whereas stenohaline fishes are osmotically more conservative with a limited ability to tolerate sudden or wide variations in environmental salinity. In teleosts, the ability to cope with differences in the environmental salinity, varies from species to species. While the vast majority of teleosts are endemic to either fresh water or sea water showing limited salinity tolerance, a few species are able to tolerate wide fluctuations in the environmental salinities and include both migratory as well as non-migratory forms. A perusal of literature reveals that the major bulk of data on the osmoregulatory physiology of fishes relates to the euryhaline species, possibly due to their remarkable capacity to adapt successfully to media of diverse salinities. Most of the extant stenohaline teleosts have been investigated only sporadically. Notable examples being goldfish, *Carassius auratus* (Houston and Mearow, 1982; Paxton and Umminger, 1983; Paxton, *et al.*, 1984), the channel catfish, *Ictalurus punctatus* (Furspan, *et al.*, 1984, Davis *et al.* 1985), some species of the genus *Serranus* (Motais, *et al.*, 1965, 1966, 1969), the barred surfperch, *Amphistichus argenteus* (Holmes and Lockwood, 1970), common carp, *Cyprinus carpio* (Hegab and Hanke, 1984), catfish, *Clarias batrachus* (Nayyar, 1992), and Atlantic cod, *Gadus morhua* (Provencher *et*

al., 1993). Of the nearly 2200 species inhabiting Indian subcontinent (Das and Kapoor, 1996), osmoregulatory physiology of not even a single species has been investigated in detail. Although the basic osmoregulatory mechanism of the catfish, *Heteropneustes fossilis*, has been worked out (Parwez, 1982), much still remains to be elucidated. This existing lacuna of knowledge in this field prompted us to undertake studies on this fish species in order to create a model for understanding the physiological basis of osmoionic *regulation of the catfish, H. fossilis*.

ACKNOWLEDGEMENTS

It is indeed a great pleasure to acknowledge my humble sense of gratitude to my supervisor, Dr. Iqbal Parwez - a teacher *par excellence,* whose detail oriented guidance, untiring encouragement and healthy criticism were a guiding light throughout the course of this investigation. My privileged association with him for his unbound interest in scientific research and analytical approach to various problems has benefited me in a manner I had never even imagined.

I am also thankful to Chairman, Department of Zoology, for extending all the help and facilities to carry out this work.

I would be failing in my duty if I do not acknowledge the help rendered by Prof. Wajih A. Nizami, Dr. Asif A. Khan and their research students, as the provision of laboratory facilities was an essential element of this investigation.

I am highly appreciative of the unbound cooperation and sincere advice extended by my lab colleagues Alka, Meenu, Zakir, Shakeel, Nicky and Arshad. Thanks are also due to Dr. Hina Parwez for her friendly attitude and encouragement.

My friends Nasreen, Nazura, Dr. Shabana, Rana, Noorus Sabah,

Nikhat and Shoeba deserve a special mention for their ever-willing help and valuable suggestions during the preparation of my thesis.

Last, but not the least, my heartfelt thanks are due to my parents and other family members without their keen cooperation this work could not have been completed. The names of younger members are worth the mention, where my brother Shariq, nephew Faisal and nieces Subuhi and Farheen appear all set to bear the mantle of the rich family tradition of seeking knowledge.

Thanks are also due to Council of Scientific and Industrial Research, New Delhi for providing financial assistance.

Fauzia A Sherwani

FAUZIA ANWAR SHERWANI

Point-wise details of the suggested changes as mentioned in the comment sheet of the foreign examiner are given below.

General comments

1. While it is appreciated that inclusion of the scientific name of the fish in the title will make it more comprehensive, it could not be done at this stage for some technical reasons since, as per rules of the university, any change in the original title needs the approval of two committees and the entire formalities take nearly one year. However, title will be suitably amended at the time of publication of the data.

2. All the tables have been deleted and only figures have been retained.

3. Pre-transfer values have been incorporated in the figures of transfer experiments.

4. All changes suggested in the text have been incorporated.

5. Many very old references, unless very important, have been pruned. The number of the references got reduced from 294 to 222.

Specific Comments

Chapter I

1. Materials and Methods have been rewritten as per suggestions.

2. More detailed description of the cortisol RIA has been given and results of the validation of assay to show parallelism, recovery, inter- and intra-assay variations and specificity have been included. Present method does not necessitate extraction of plasma before doing RIA.

Results

1. All deletions and additions mentioned in the thesis in this chapter have been incorporated.

Chapter II

Even though the difference between 30% and 35% SW may appear to be small but the results are what they are.

Results and discussion

1. The interpretations of results in the discussion part as pointed out on page 41, 45 and 46 in the original thesis have been modified. Similarly, concluding results on page 47 have been suitably changed.

2. Discussion has been re-written as per suggested lines comparing plasma osmolarity, glucose, liver and muscle glycogen changes between Experiments 1 & 2.

3. All the comments written on the thesis have been incorporated.

Chapter III

Materials and Methods

1. Experimental protocol has been put as first item in Materials and Methods.

2. As mentioned in the text, Tagawa and Hirano (1987) method has been followed for T_4 RIA. Rest of the details are same as described in the text.

3. Dilution factor of the plasma in T_4 parallelism has been incorporated in the figure.

Discussion

1. Corrections regarding salinity figures quoted in Kultz et al (1992) have been made.

2. Corrections regarding Hwang et al (1989) salinity figures have been made.

3. Discussion with regard to role of F has been modified.

4. Statement regarding T_4 has been modified.

5. Comments written in the thesis have been incorporated.

Chapter IV

All comments written in the thesis have been incorporated.

CHAPTER I

EFFECT OF STRESS AND NUTRITIONAL STATE ON THE CATFISH, *HETEROPNEUSTES FOSSILIS*

INTRODUCTION

Stress is known to induce physiological imbalance of varying degree within the entire body of all the animals including fishes (Donaldson, 1981; Patino *et al.*, 1986; Vijayan and Leatherland, 1990; Vijayan and Moon, 1992, 1994; Nielsen, *et al.*, 1994; Knoph, 1995; Einarsdóttir and Nilssen, 1996). Various situations causing stress include major and sudden changes in the environment such as electroshocking (Schreck *et al.*, 1976); a rapid change in the temperature (Jürss *et al.*, 1984a; Finstad *et al.*, 1988, 1989; Finstad and Thomassen, 1991; Schwarzbaum *et al.*, 1991, 1992; Staurnes *et al.*, 1994; Ottolenghi, *et al.*, 1995) and salinity fluctuations (Jackson, 1981; McKay and Gjerde, 1985; Hutchinson and Hawkins 1990; Mayer-Gostan and Noan. 1992; Soengas *et al.*, 1993, 1995a,b; Gaumet *et al.*, 1995; Woo and Chung, 1995; Sayer and Reader, 1996). There are also evidence to show that minor disturbances like aquarium transfer (Chavin and Young, 1970) affect the fish. Moreover, various

routine laboratory and experimental methods such as injection (Umminger, 1973); handling (Barton *et al.*, 1980, 1986; Waring *et al.*, 1992; Biron and Benfey, 1994) and anaesthetization (Davis *et al.*, 1982; Laidley and Leatherland, 1988; Spottle *et al.*, 1991; Knoph and Olsen 1994; Knoph, 1995) are known to be stressful to the fishes.

Teleosts under stress exhibit an initial neuroendocrine response which leads to the release of catecholamines from the chromaffin cells and cortisol (F) from the head kidney (Vijayan and Moon, 1994). This initial hormonal response is known as "primary response" to stress. The primary response leads to relatively more prolonged secondary response which can bring about several metabolic changes (Vijayan and Moon, 1992) and the hydromineral imbalance like changes in plasma electrolyte contents, hematocrit value and plasma osmolarity (Donaldson, 1981; Wedemeyer *et al.*, 1990; Barton and Iwama, 1991; Lowe and Wells, 1996).

Various strategies have been proposed to counter stress caused by routine laboratory manipulations. While some researchers have reduced the effect of stress by the "training procedure" (Slicher *et al.*, 1966: on killifish, *Fundulus heteroclitus;* Rush and Umminger, 1978: on goldfish, *Carassius auratus*) during which the fish are frequently and consistently subjected to moderate handling stress during

feeding and replenishment of water in the aquaria, others eliminated the stress-induced changes by quickly capturing and sacrificing the fish (Chavin and Young, 1970: on goldfish, *Carassius auratus*). Stress could also be reduced by placing the fish in 100 mM NaCl solution (Wedemeyer, 1972). However, the method which is used most commonly is the anaesthetization of the fish before blood sampling which may otherwise lead to increased plasma F (Thomas and Robertson, 1991; Knoph and Olsen, 1994; Knoph, 1995). One of the most commonly used anaesthetic is MS222 (ethyl m-aminobenzoate methanesulphonate), although a variety of others like quinaldin sulfate (Davis *et al.*, 1982); metomidate (Knoph, 1995); benzocaine (Laird and Oswald, 1975; Booke *et al.*, 1978) and 2 phenoxy ethanol (Cornell *et al.*, 1994) are also used.

Apart from handling stress, nutritional status is likely to influence the osmoregulatory physiology of the fish. Many species of fish have remarkable ability to live without food for long durations (Loughna and Goldspink, 1984; Machado *et al.*, 1988; Collins and Anderson, 1995). In this regard, the male silver eel can survive for about 1515 days (more than 4 years) at 15^{o}C without food and during this period it had lost not less than 76% of its initial weight (Love, 1970). As a result of different life style with

respect to the habitats, the mobilization of energy reserve varies among fishes (Alliot *et al.*, 1984). Among other things like diet composition (Machado *et al.*, 1988)); endogenous rhythms such as reproductive cycle (Love, 1970) or other environmental effects such as temperature (Pastoureaud, 1991) and salinity (Woo and Mürat, 1981; Jürss *et al.*, 1983, 1986; Roche *et al.*, 1989; Soengas *et al.*, 1995a, b), the depletion of energy reserves may also be related to the nutritional status of the fish and the energy demand in its milieu (Jürss *et al.*, 1984b; Foster *et al.*, 1993). Moreover, there are evidence to show that energy deficiency due to cessation of feeding may lead to disorders in various osmoregulatory parameters such as plasma osmolarity/plasma electrolyte (Jürss *et al.*, 1982a, 1984b; 1987; Nance *et al.*, 1987; Kültz and Jürss, 1991) and in major carbohydrate fuels like plasma glucose, liver and muscle glycogen (Renaud and Moon, 1980; Black and Love, 1986; Lim and Ip 1989; Sheridan and Mommsen, 1991; Collins and Anderson 1995). Therefore, while performing any study dealing with osmoregulatory physiology, it is of utmost importance that the experimental animal should be in a nutritionally sound state. For this purpose, the adequate time interval of feeding during acclimation as well as experimental period has to be decided with great care. It should be such that the

energy/nutritional requirements are met in most efficient manner, so that the animal is in a stress-free state from the nutritional point of view.

Therefore, the present study has twin objectives:

(a) To evaluate the effect of handling stress on the catfish by monitoring the changes in the plasma F, an indicator of primary response, and the plasma osmolarity which is a hematological indicator of secondary response.

(b) To study the nutritional state of the catfish following cessation of feeding by assessing changes in the major carbohydrate fuels such as plasma glucose, liver and muscle glycogen and plasma osmolarity.

MATERIALS AND METHODS

1. Collection and care of fish :

Catfish, *Heteropneustes fossilis* were obtained from the local fish market of Aligarh. They were kept in glass aquaria (60 X 25 X 30 cm) containing dechlorinated tap water (TW) and the lighting schedule of 12 hr of light (08:00 to 20:00 hr) alternating with 12 hr of darkness (20:00 to 08:00). Fish (body weight 35-60 g) were acclimated to laboratory conditions for 15 days prior to initiation of

experiments. During this period, they were fed *ad libitum* daily with Hindlever laboratory animal feed (Hindustan Lever Limited, Bombay, India) and the water in the aquaria was renewed daily with aged TW adjusted to the laboratory conditions.

2. Experiment 1

Effect of stress and anaesthetization on plasma profiles of osmolarity and F of the catfish, *H. fossilis*:

a. Treatments

Fish were allocated into three groups each with 4-9 specimens and were kept in glass aquaria containing 20 liters of TW. Fish from the first group were gently netted from the aquarium and the blood was collected from the caudal artery without anaesthesia as described below. In the second group, the blood samples were obtained from the fish anaesthetized with MS222 at a dose of 100 mg/l (This concentration of MS222 is enough to immobilize the fish within 1 min). While in the third group, the blood was drawn without anaesthesia after giving 2-3 min stress by vigorously chasing the fish with the hand net. Plasma osmolarity and plasma F levels were analyzed according to the methods described below.

b. Blood collection and plasma separation:

The blood samples were taken as rapidly as possible (in less than 2 min) with or without anesthesia. Blood samples were obtained from the caudal artery using heparinized Glass Van syringes fitted with 24 gauge disposable needles. Immediately after collection, the blood was centrifuged for 10 min at 3000 rpm (Remi Ltd., India, model no. R8C)) and plasma separated and stored at -20^{o}C until analyzed. In all the subsequent experiments described in Chapters II-IV, blood was collected from non-anaesthetised fish.

c. Determination of plasma osmolarity:

Plasma osmolarity was determined by Vapour Pressure Osmometer (Wescor 5500, Utah, USA).

The instrument was initially calibrated with 100, 290 and 1000 mmol/kg standards and this calibration was checked frequently during estimations. For sample estimation, 10 µl was placed on sample disc with the help of micropipettor and the estimation was done following the manufacturer's manual.

d. F radioimmunoassay :

F levels in plasma were measured by radioimmunoassay (RIA) using Biodata Cortisol Bridge Kit (code 14394 manufactured by Biodata S.P.A. Montecelio). The kit has been designed for quantitative determination of F in plasma

samples by RIA without extraction (direct method).

Working procedure:

Cortisol Bridge Coated Tubes (antibody coated tubes) were stored either at room temperature or at 4°C and Cortisol Bridge Standards were stored at 4°C. Standards were used directly without any reconstitution.

Reconstitution of other kit reagents:

^{125}I-Cortisol Bridge - The freeze-dried powder was added into the solution by pushing the red cap and mixed gently until its complete solubilization. The solution was used 30 min after reconstitution of tracer.

Serotest S - The contents of the vial were reconstituted with 1 ml double distilled water (DDW) and were mixed gently until the complete solubilization of freeze-dried powder. The reconstituted reagent may be stored at 4°C up to two days before use and may be kept for longer duration at -20°C.

Test procedure:

The reagents were allowed to reach room temperature and mixed gently before use. For the assay, cortisol Bridge Coated tubes (antibody coated tubes) were set in duplicate for each standard including Bo (zero standard concentration), serotest and samples. Fifty µl of sample/standard/serotest

were pipetted into the respective tubes. Into these tubes as well as total radioactivity tubes, 1.0 ml of ^{125}I-Cortisol was pipetted. They were mixed gently using side to side motion (enough to ensure well mixing) and incubated for 90 min at 37^{o}C in water bath. The fluid was aspirated and the radioactivity was counted in gamma counter (ECIL, Hyderabad, India) for 1 min. For total radioactivity determination, the tubes were counted after tracer addition and before aspiration step. The mean radioactivity of these tubes was considered as total radioactivity.

Per cent relative binding of each of standard, sample and Serotest was calculated with the following equation -

$$\frac{\text{Mean counts (standard, sample or serotest)}}{\text{Bo mean counts}} \times 100 = \% \text{ relative binding}$$

The dose response curve was drawn on a semi-logarithm paper by plotting the % relative binding of each standard (y-axis) against the relative concentration (x-axis) and F concentration in plasma samples was obtained by interpolation (Fig. 1). F concentration was expressed as ng/ml plasma.

F RIA Validation:

F RIA validation details are as follows

(i) Sensitivity:

Assay sensitivity was 10 ng/ml.

(ii) Precision:

The intraassay and interassay coefficients of variation were 3.6% (n = 15) and 6.7% (n = 10) respectively.

(iii) Parallelism:

Serial dilutions of plasma (50, 12.5, 3.1 µl) from *H. fossilis* exhibited inhibition curve parallel to the standard curve (Fig. 1). Each sample was assayed in duplicate and average values were plotted.

(iv) Recovery:

Recovery test performed by adding to a known plasma sample different concentrations of F standards (25, 50, 250 and 500 ng/ml) gave following results.

$Y = -1.0797 + 1.006X$

Correlation Coefficient (r) = 0.9999

(v) Specificity:

Data on cross reactivity of F antiserum with various steroids (Based on information provided by Biodata S.P.A. Montecelio) is as follows:

Cross-reacting steroids	Cross reaction (%)*
F	Taken as 100
Deoxycorticosterone	0.1
11-Deoxycortisol	8.6
Cortisone	2.5
Prednisone	1.8
Corticosterone	1.7
17 ∝-OH-Progesterone	0.7
Progesterone	absent up to 100 µg/ml
Dexamethasone	absent up to 500 µg/ml
Aldosterone	absent up to 50 µg/ml
Dihydrocorticosterone	absent up to 100 µg/ml
Spironolactone	absent up to 100 µg/ml

* Percentage cross-reaction calculated at B/B_o of 50%

3. Experiment 2:

Changes in plasma osmolarity and glucose and liver and muscle glycogen contents following cessation of feeding of the catfish, *H. fossilis*:

a. Treatments

The catfish were either fed *ad libitum* daily in the morning (control group) or feeding was terminated (experimental group) for 2, 3, 6, 10 and 15 days and then various tissue samples (blood, liver and muscle) were obtained from each group containing 4-5 fish. Thus, five above mentioned groups were starved for 2, 3, 6, 10 and 15 days prior to sampling. A control group was sampled separately with each time point. The blood was collected from the caudal artery as described before and the plasma thus obtained was utilized for the estimation of plasma osmolarity and the glucose according to the methods described in this chapter. The fish was decapitated quickly with least stress and a small piece of muscle was excised from just beneath the caudal fin and liver was dissected out to estimate glycogen content according to the method described below.

b. Blood collection and plasma separation:

The procedure for blood sampling and plasma separation was similar as described earlier. The blood was collected without anaesthesia.

c. Plasma osmolarity determination:

Plasma osmolarity was determined according to the method described earlier.

d. Plasma glucose determination:

Plasma glucose was assayed by the glucose-O-toluidine method (Hyvarinen and Nikkila, 1962). To 0.1 ml of plasma sample and each glucose standard concentrations (range 3-12 mmol/l), 3 ml of O-toluidine reagent (1.5 g of thiourea in 940 ml of glacial acetic acid, with the subsequent addition of 60 ml of O-toluidine when completely dissolved) was added. All the tubes were stoperred and incubated at 100^{o}C in water bath for 12 min. After cooling these tubes at room temperature, the optical density (O.D.) was measured at 630 nm against reagent blank. The standard graph was drawn and the plasma glucose concentrations obtained in mmol/l were ultimately converted to mg%.

e. Liver and muscle glycogen determination :

Glycogen, both in muscle and liver was estimated by Anthrone Method (Roe and Dailey, 1966). The tissue was washed in 0.6% cold saline, quickly blotted between folds of moistened filter paper and promptly weighed (wet). The tissue was chopped thoroughly followed by the addition of appropriate volume of 5% trichloroacetic acid (TCA) solution (2-3 ml/g). This was thoroughly homogenized (in cold) in a

motor driven homogenizer (York Scientific Industries, Delhi, India) employing nearly ten strokes. The homogenate was then centrifuged at 3000 rpm for 10 min in a high speed refrigerated centrifuge (Indian Equipment Corporation, Model IEC-25-Portomin Deluxe). The sediment was re-homogenized with half of the volume of TCA previously added and again centrifuged in cold. To the combined supernatants or filtrates twice the volume of 45% ethanol was added and after mixing well, it was left in a refrigerator overnight which helped in precipitating out the glycogen. The precipitate was collected by centrifugation. The precipitate was washed with ethanol and then with ethyl ether before storing in a desiccator.

Different volumes (range 0-100 µg) of standard glucose solution (10 mg/100 ml) were pipetted into a series of test tubes and made the volume to 1 ml with DDW. Dried precipitates of glycogen (unknown samples) were also dissolved in 1 ml of DDW. Four ml of Anthrone Reagent (0.2% in concentrated H_2SO_4) was added to each tube and mixed well. The tubes were kept in boiling water bath for 10 min. After cooling them to room temperature, O.D. was taken at 620 nm using a tube containing 1 ml DDW and 4 ml reagent as blank. The glycogen concentration was derived from the following equation -

$$\text{Glycogen Concentration} = \frac{\text{Standard Glucose Concentration}}{1.11} \times \frac{E_{620} \text{ of Glycogen Concentration}}{E_{620} \text{ of Glucose Concentration}}$$

where E_{620} glycogen and E_{620} glucose is the O.D. of glycogen and glucose at 620 nm of the standard glucose concentration.

4. Statistical Analysis:

Data for all parameters were expressed as mean ± standard error. Statistical comparisons between experimental and control groups were made by Student's *t*- test (Snedecor and Cochran, 1971).

RESULTS

Experiment 1:

Plasma F:

There was no significant difference in the plasma F concentration when the blood was collected from fish with or without anaesthetization and when the fish were subjected to stress (Fig. 2).

Plasma osmolarity :

Plasma osmolarity were not significantly different among the three groups (Fig. 3).

Experiment 2:

Plasma osmolarity:

Cessation of feeding up to 15 days did not significantly change plasma osmolarity of the catfish. However, there was a decreasing trend up to 6 days of cessation of feeding beyond which the values became almost similar to that of fed control (Fig. 4).

Plasma glucose:

Cessation of feeding up to 15 days did not show significant effect on the plasma glucose profile which remained more or less constant (Fig. 5).

Liver glycogen:

There was a significant decline in the glycogen content of liver during the cessation of feeding. The values were most significantly lowered from 2 to 6 days of cessation of feeding ($P<0.001$), showed some increase on day 10, but still significantly lower ($P<0.05$) compared to fed control. However, the levels became nearly equal to the control after 15 days of cessation of feeding although they were still at lower side (Fig. 6).

Muscle glycogen:

The glycogen content in muscle showed a significant decline up to 6 days of cessation of feeding ($P<0.025$) (Fig. 7). The parity with fed control was obtained from 10 days onwards.

DISCUSSION

It is apparent that routine laboratory handling does not cause any appreciable stress on the catfish, *H. fossilis* as evidenced from no significant difference in plasma F levels between non-anaesthetized and anaesthetized groups. This corroborates the findings of Strange and Schreck (1978) on yearling chinook salmon, *Oncorhynchus tshawytscha* and those of Laidley and Leatherland (1988) on rainbow trout, *Salmo gairdneri* where they observed no significant elevation in the F concentration in anaesthetized and non-anaesthetized groups. However, Wedemeyer (1970) observed that anaesthetization with MS222 at a dose of 80 mg/l induced a progressive depletion of interrenal ascorbate levels implying that the anaesthetization was a physiological stressor in this fish. It is likely that the stress susceptibility of the catfish is lesser than that of the rainbow trout, *Salmo gairdneri* (Wedemeyer, 1970). This is also borne out by the observation when sustained stress due to continuous chasing

of the catfish with a hand net for 2-3 min failed to evoke any significant rise in plasma F (group III, Fig. 2). That a mild handling stress does not cause any significant change in the plasma levels of F has also been observed by a number of other investigators. For instance, goldfish, *Carassius auratus* did not exhibit any change in plasma F levels until 10 min of mild restraint (Spieler, 1974) and until 15 min after exposure to a handling disturbance (Fryer, 1975). Barton *et al.*, (1980) observed that serial removal of cohorts of rainbow trout, *Salmo gairdneri* from the same aquarium also did not cause any significant change in plasma F concentration in remaining fish. On the other hand, many studies have shown that even the disturbances of minor magnitude such as removal of fish from aquaria or a person entering the room housing the aquaria can elevate plasma F levels (Pickering *et al.*, 1982; Pickering and Pottinger, 1983; Barton *et al.*, 1985). This clearly suggests that the degree of susceptibility to stress is a species-specific phenomenon.

Anaesthetization and handling stress did not affect plasma osmolarity (one of the hematological indicator of secondary response) of the catfish. This further substantiates the hardy nature of the catfish and is in keeping with similar observations made by Knoph (1995) on

Atlantic salmon, *Salmo salar*. However, according to Wedemeyer, *et al*. (1990) the usual ionic response to stress by fish in fresh water is a decline in plasma osmolarity resulting from an osmoregulatory imbalance. Likewise, other reports show disturbance of salt and water balance during anaesthetization (Houston *et al*., 1971; Sovivo *et al*., 1977; Korcock *et al*., 1988). The present results establish that the catfish, *H. fossilis*, is fairly hardy and is not susceptible to stress by routine laboratory handling.

The present study also demonstrates that suspension of feeding for up to 15 days does not show any effect on the plasma glucose levels and osmolarity of the catfish, *H. fossilis*. The unchanged plasma osmolarity following cessation of feeding have also been reported in tilapia, *Oreochromis mossambicus* (Kültz and Jürss, 1991). While the results of present study showing constant plasma glucose following suspension of feeding are in accordance with those reported in *Esox lucius* (Ince and Thorpe, 1975), *Ophiocephalus maculatus* (Woo and Cheung, 1980), *Limanda limanda* (Fletcher, 1984), they differ from those reported in *Notopterus notopterus and Anguilla anguilla*, where blood glucose levels exhibit change after cessation of feeding (Narasimhan and Sundararaj, 1971; Larsson and Lewander, 1973; Dave *et al*., 1975).

The sustained plasma glucose levels in unfed catfish, *H. fossilis* may be either due to gluconeogenesis (Cowey and Sargent, 1979) or glycogenolysis (Narasimhan and Sundararaj, 1971). Our data has shown that the termination of feeding induced highly significant decline in liver glycogen from 2 to 10 days beyond which the values became similar to the fed control. The sustained blood glucose levels without any dietary input may possibly be due to the breakdown of liver glycogen into glucose *i.e.* hepatic glycogenolysis which has also been shown in *Notopterus notopterus* (Narasimhan and Sundararaj, 1971); juvenile perch, *Perca fluviatilis* (Mehner and Wieser, 1994); golden perch, *Macquaria ambigua* (Collins and Anderson, 1995). The present data, however, differ from those reported in snakehead, *Ophiocephalus maculatus* (Woo and Cheung, 1980); mudskipper, *Boleophthalmus boddaerti* (Lim and Ip, 1989); coho salmon, *Oncorhynchus kisutch* (Sheridan and Mommsen, 1991) where no significant decrease in the liver glycogen was observed after fasting. Possibly the maintenance of glycogen in the liver of these animals is due to active gluconeogenesis (Moon and Johnston, 1980; Black and Love, 1986; Sheridan and Mommsen, 1991).

We have also observed a significant decline in the muscle glycogen levels from 2 to 6 days following cessation of feeding after which the levels obtained parity with fed

control. There was also a trend towards decrease in the levels of muscle glycogen after cessation of feeding in *Boleophthalmus boddaerti* (Lim and Ip, 1989), *Perca fluviatilis* (Mehner and Wieser, 1994); *Macquaria ambigua* (Collins and Anderson, 1995). The simultaneous decrease in muscle and liver glycogen up to 6 days of suspended feeding in the catfish may presumably indicate that hepatic and muscle glycogenolysis proceed simultaneously and that during the early period of suspended feeding catfish draws its energy from both tissues. This agrees with the observations of Black and Love (1986) and Mehner and Wieser (1994) where liver and muscle glycogen decline occur simultaneously. Hence, present study suggests that cessation of feeding affects carbohydrate metabolism of the catfish and that it should be fed continuously during acclimation and experimental period. However, feeding may not be critically important in osmoregulatory studies.

Fig. 1. Standard curve for cortisol showing parallelism with serial dilutions of plasma from catfish. B/Bo is per cent relative binding. Each point represents the average of duplicate determinations.

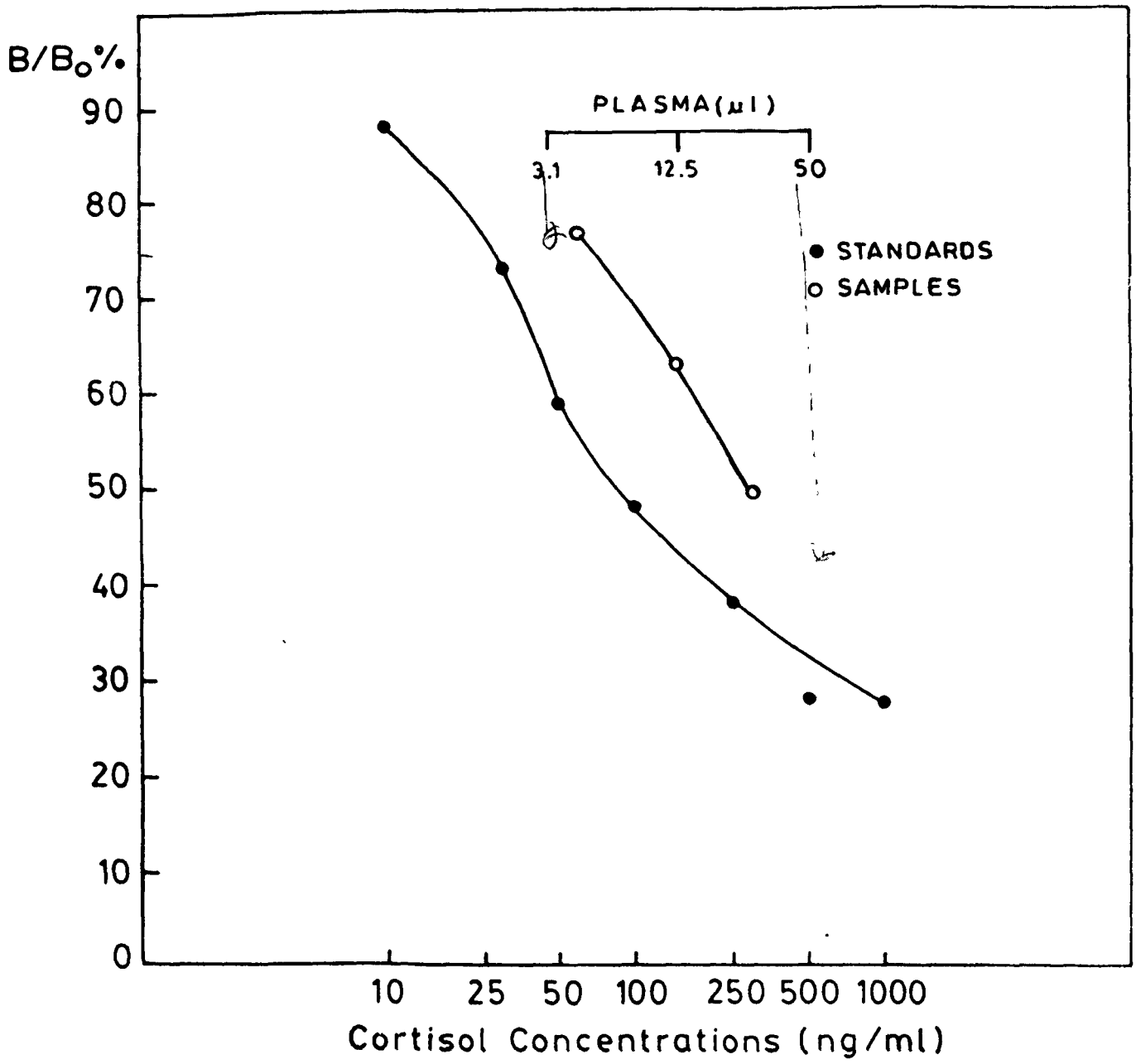
B/B₀%
90
80
70
60
50
40
30
20
10
0
PLASMA(µl)
3.1
12.5
50
● STANDARDS
o SAMPLES
10
25
50
100
250
500
1000
Cortisol Concentrations (ng/ml)

Fig. 1

Fig. 2. Changes in plasma cortisol in anaesthetized, non-anaesthetized and stressed catfish, *Heteropneustes fossilis*. The comparison has been made with non-anaesthetized group.

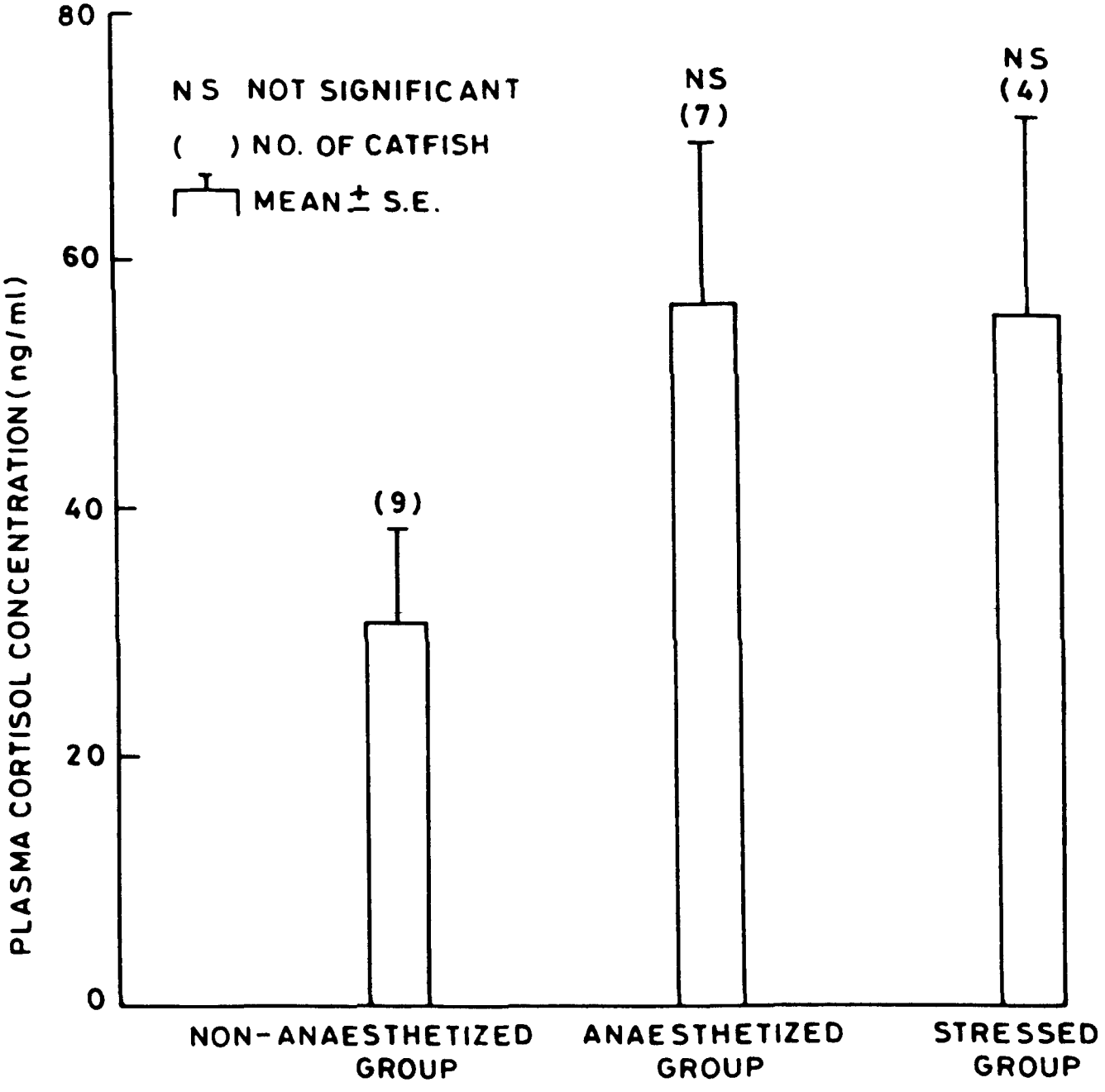
80
60
40
20
0
PLASMA CORTISOL CONCENTRATION (ng/ml)
N S NOT SIGNIFICANT
( ) NO. OF CATFISH
MEAN ± S.E.
(9)
NS
(7)
NS
(4)
NON-ANAESTHETIZED GROUP
ANAESTHETIZED GROUP
STRESSED GROUP

Fig. 2

Fig. 3. Changes in plasma osmolarity in anaesthetized, non-anaesthetized and stressed catfish, *Heteropneustes fossilis*. The comparison has been made with non-anaesthetized group.

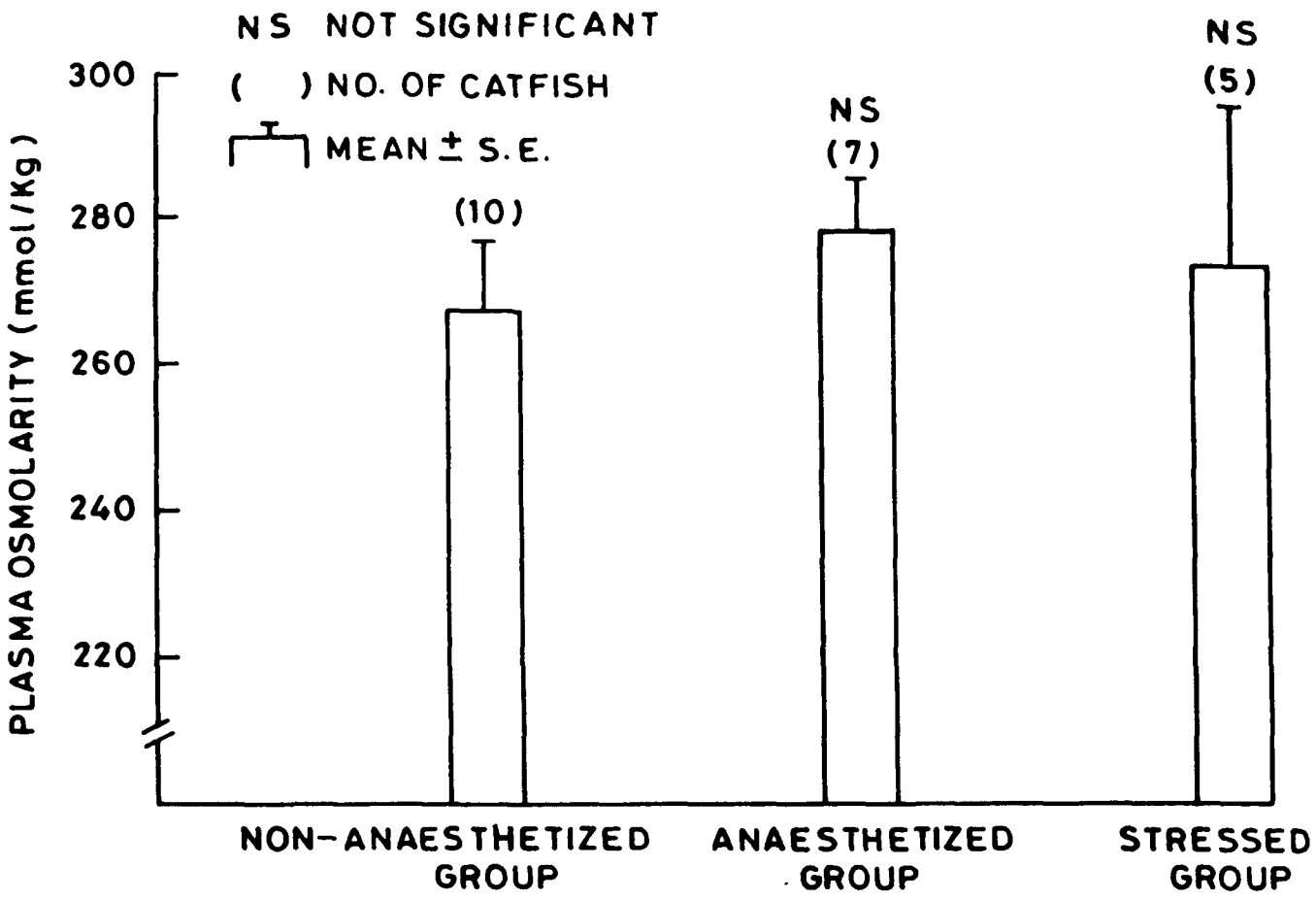
NS NOT SIGNIFICANT
( ) NO. OF CATFISH
MEAN ± S.E.
PLASMA OSMOLARITY (mmol/Kg)
300
280
260
240
220
(10)
NS
(7)
NS
(5)
NON-ANAESTHETIZED GROUP
ANAESTHETIZED GROUP
STRESSED GROUP

Fig. 3

Fig. 4. Changes in plasma osmolarity following cessation of feeding of the catfish, *Heteropneustes fossilis*. The comparison has been made with control group.

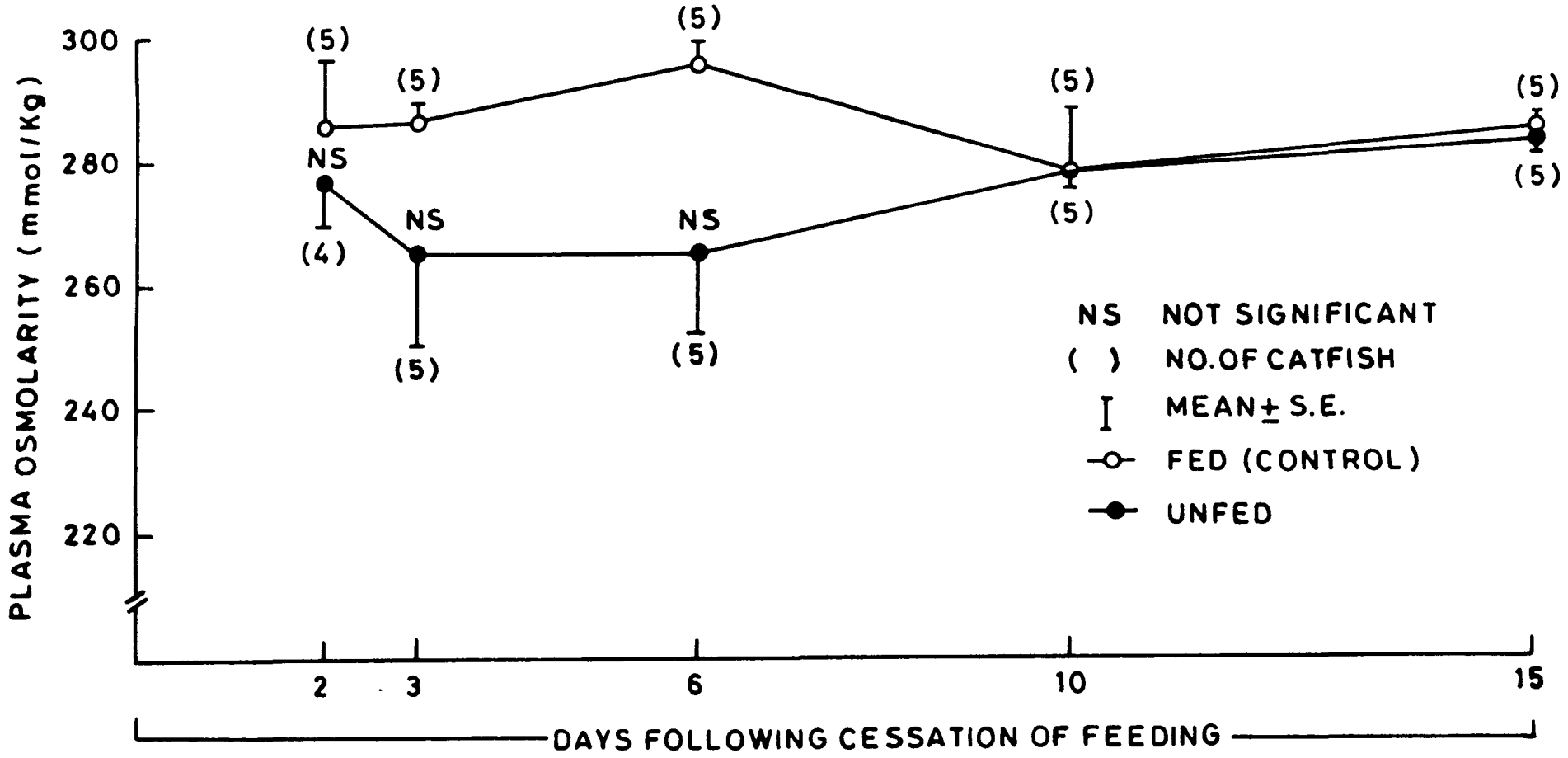
PLASMA OSMOLARITY (mmol/Kg)
300
280
260
240
220
(5)
(5)
(5)
(5)
(5)
(5)
NS
NS
NS
(4)
(5)
(5)
(5)
NS NOT SIGNIFICANT
( ) NO.OF CATFISH
MEAN ± S.E.
FED (CONTROL)
UNFED
2
3
6
10
15
DAYS FOLLOWING CESSATION OF FEEDING

Fig. 4

Fig. 5. Changes in plasma glucose concentration following cessation of feeding of the catfish, *Heteropneustes fossilis*.

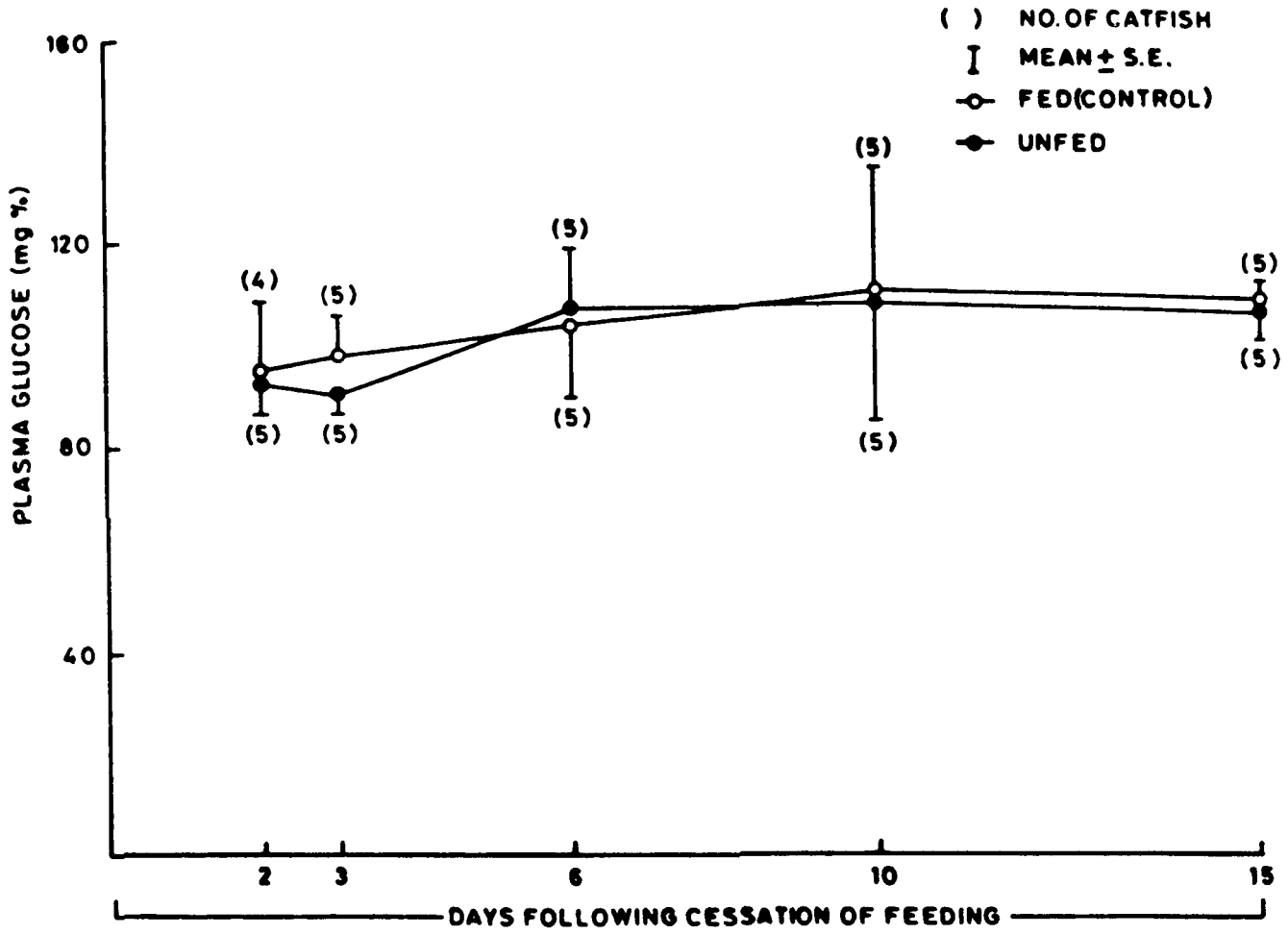
( ) NO. OF CATFISH
MEAN ± S.E.
FED(CONTROL)
UNFED
PLASMA GLUCOSE (mg %)
160
120
80
40
(4)
(5)
(5)
(5)
(5)
(5)
(5)
(5)
(5)
2
3
6
10
15
DAYS FOLLOWING CESSATION OF FEEDING

Fig. 5

Fig. 6. Changes in liver glycogen concentration following cessation of feeding of the catfish, *Heteropneustes fossilis*. Asterisks denote significant difference in values compared to control group.

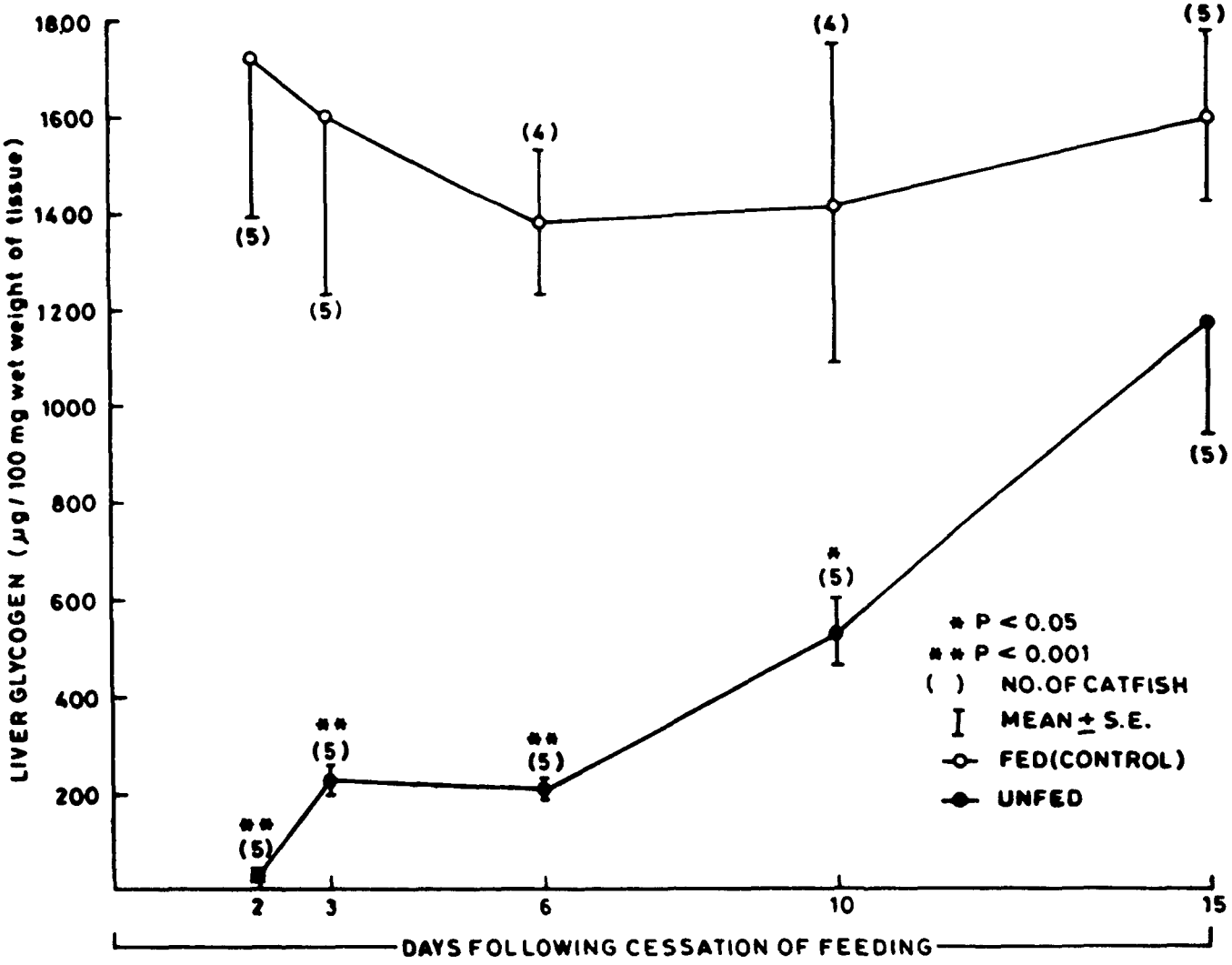
LIVER GLYCOGEN (µg/100 mg wet weight of tissue)
1800
1600
1400
1200
1000
800
600
400
200
(5)
(5)
(4)
(4)
(5)
(5)
**
(5)
**
(5)
**
(5)
*
(5)
2
3
6
10
15
DAYS FOLLOWING CESSATION OF FEEDING
* P < 0.05
** P < 0.001
( ) NO. OF CATFISH
MEAN ± S.E.
FED(CONTROL)
UNFED

Fig. 6

Fig. 7. Changes in muscle glycogen concentration following cessation of feeding of the catfish, *Heteropneustes fossilis*. Asterisks denote significant difference in values compared to control group.

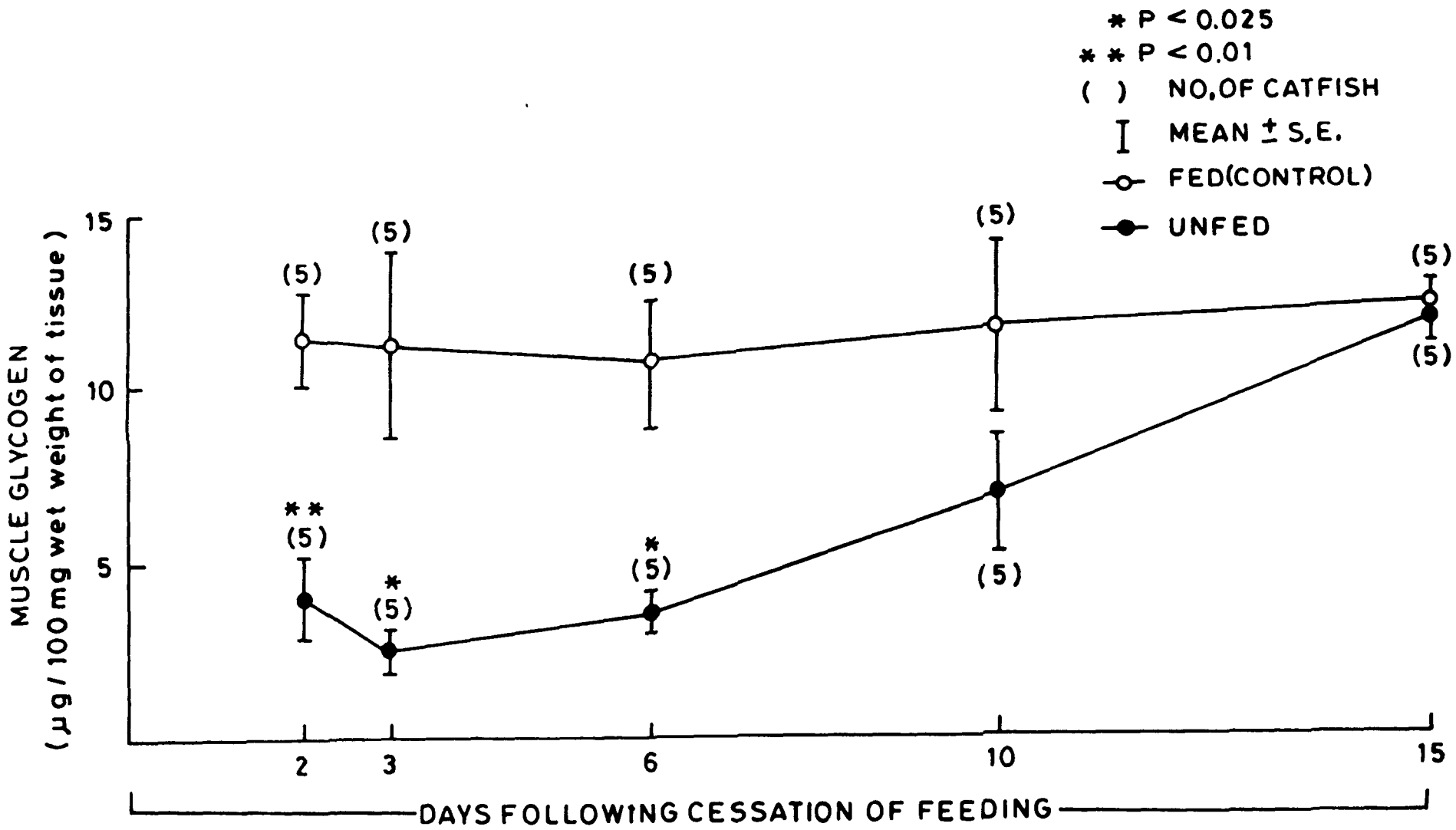
* P < 0.025
** P < 0.01
( ) NO. OF CATFISH
I MEAN ± S.E.
FED(CONTROL)
UNFED
MUSCLE GLYCOGEN
(μg / 100 mg wet weight of tissue)
15
10
5
(5)
(5)
(5)
(5)
(5)
(5)
**
(5)
*
(5)
*
(5)
(5)
(5)
2
3
6
10
15
DAYS FOLLOWING CESSATION OF FEEDING

Fig. 7

CHAPTER II

CARBOHYDRATE PROFILES DURING OSMOTIC ADJUSTMENTS OF THE CATFISH, *HETEROPNEUSTES FOSSILIS*

INTRODUCTION

Acclimation of the fish to higher external salinity entails high energy demand because of the increased active transport rate of ions in various osmoregulatory organs such as the gills, kidney and/or intestine (Evans, 1984; Febry and Lutz, 1987; Kirschner, 1993).

Most studies on the relationship between energetic and osmoregulatory metabolism are based on the estimates of oxygen consumption of the whole animal (Furspan *et al.*, 1984; Febry and Lutz, 1987; McCormick *et al.*, 1989, Morgan and Iwama, 1991). Several of these show an increase in the metabolic rate as a result of ambient salinity alterations. For example, a metabolic rate increase of 1-2% in Atlantic salmon, *Salmo salar* L. (McCormick *et al.*, 1989), 20% in cichlids (Febry and Lutz, 1987) and rainbow trout, *Oncorhynchus mykiss* (Walbaum) (Morgan and Iwama, 1991) and even 50% in catfish, *Ictalurus nebulosus* Lesuear (Furspan *et al.*, 1984) have been reported following their transfer to higher salinities. Nevertheless, the scientific data do not

suggest a clear-cut direct relationship between salinity-related changes in whole body metabolism *vis-a-vis* changes in the salt and water metabolism *i.e.* osmoregulation (Febry and Lutz, 1987; Morgan and Iwama, 1991; Kirschner, 1993).

One of the approaches to study the energetic aspect of osmoregulation is to analyse the alterations in the profiles of substrates of energy metabolism. The studies in the above area are somewhat limited (McCormick and Saunders, 1987) and inconclusive, although some conclusive data on lipid metabolism *vis-a-vis* osmoregulation have been reported (Sheridan, 1989).

Since carbohydrates are utilized easily and have high ATP production rate, it is worthwhile to study the changes in the carbohydrate metabolism during osmo-ionic adjustments of the fish. Moreover, the liver and muscle are directly associated with the formation of glucose through glycogenolysis (the breakdown of glycogen) or gluconeogenesis, thus, it is more logical to investigate the modifications of carbohydrates during osmoregulation in the above organs (Saurez and Mommsen, 1987). There are evidence showing highly significant changes in the profiles of carbohydrates when the fish are acclimated in different salinities. Glucose may either increase (Hegab and Hanke,

1984, 1986; Madsen, 1990a; Nayyar, 1992; Soengas *et al.*, 1995a), decrease (Bashamohideen and Parvatheswararao, 1972; Farghaly *et al.*, 1973) or remain unchanged (Woo and Fung, 1981). Liver and muscle glycogen mostly decreased in most fish (Woo and Murat, 1981; So and Chan, 1985; Hanke, 1991; Soengas *et al.*, 1993, 1995a), remained constant in few others (Bashamohideen and Parvatheswararao, 1972) or increase following transfer to higher salinities (Assem and Hanke, 1979a; Nayyar, 1992). These data show highly variable response pattern and no broad generalization can be made regarding metabolic pattern of fish following transfer to different salinities.

The changes occurring during transfer of the stenohaline catfish, *H. fossilis* to different salinities have been studied at both osmoregulatory and endocrine levels (Parwez *et al.*, 1979, 1984, 1994; Goswami *et al.*, 1983, Parwez and Goswami, 1985). However, no information is available on the relationship between osmoregulation and energy metabolism. In the present study, an attempt has been made to investigate the role of readily oxidisable carhohydrate, glucose, and the carbohydrate reserves, glycogen during transfer of the catfish from tap water (TW) to 30% and 35% sea water (SW) and reverse transfer from 35% SW to TW. Plasma osmolarity profile was also measured.

MATERIALS AND METHODS

Artificial SW:

Artificial SW was prepared by dissolving the following salts in dechlorinated TW: NaCl, 400.8 mM; KCl, 9.8 mM; $CaCl_2$, 10.1 mM; $MgCl_2$, 52.7 mM; Na_2SO_4, 27.8 mM; $NaHCO_3$, 2.5 mM and NaBr, 0.6 mM (Goswami *et al.*, 1983). Various dilutions of SW were prepared with dechlorinated TW.

Experiment 1:

Changes in plasma osmolarity and glucose and liver and muscle glycogen contents during osmotic adjustment of the catfish, *H. fossilis* following transfer from TW to 30% and 35% SW

Groups of catfish were transferred from TW to aquaria containing 30% (320 mmol/Kg) and 35% (360 mmol/Kg) SW and to TW to serve as control. Fish in all groups were subjected periodically to handling to eliminate stress factor during sampling. Four to five fish from each group were sampled at 3, 24 hr and 3, 6, 10 and 15 days post-transfer. Blood samples were collected in heparinized syringes from the caudal artery as described in Chapter I, centrifuged immediately and plasma was separated for measurement of osmolarity and glucose. A piece of muscle from just beneath the caudal fin and liver were excised for determining glycogen content. The data are presented in Figs. 1-4.

Experiment 2:

Changes in plasma osmolarity and glucose and liver and muscle glycogen contents of the catfish, *H. fossilis* following reverse transfer from 35% SW to TW

Catfish acclimated for 15 days in 35% SW were transferred to TW and 35% SW (control). Feeding and water replenishment in all the aquaria were done every day. Four to five fish from each group were sampled at 3, 24 hr and 3, 6, 10 and 15 days following transfer. Blood samples were obtained by the method described earlier. Plasma osmolarity, plasma glucose and glycogen content in liver and muscle were determined as described in Chapter I. The data are presented in Figs. 5-8.

RESULTS

Experiment 1:

Plasma osmolarity:

Plasma osmolarity increased significantly ($P<0.001$) 3 days after transfer of catfish in both 30% and 35% SW. The elevated levels were maintained until 15 days post-transfer. (Fig. 1).

Plasma glucose:

Transfer of catfish to 30% and 35% SW significantly

increased ($P<0.05$ and $P<0.025$ respectively) plasma glucose 3 hr after transfer (Fig. 2). Plasma glucose level decreased significantly ($P<0.05$) in 35% and 30% SW on day 1 and 3 respectively after transfer. Plasma glucose levels were almost similar in all treatments from day 10 onwards. The levels on day 6 were lower but not significantly different from TW control.

Liver Glycogen:

As shown in Fig. 3, transfer of catfish to 30% and 35% SW significantly decreased liver glycogen content from 3 hr ($P<0.025$) to 24 hr post-transfer ($P<0.001$). On day 3, the levels of liver glycogen in both groups exhibited a transient increase during which the values became almost equivalent to TW control. However, the levels significantly decreased again on day 6 ($P<0.001$) after which the parity with TW control was achieved in both groups (Fig. 3).

Muscle Glycogen:

Muscle glycogen contents significantly increased ($P<0.001$) 3 hr after transfer of catfish to 35% SW but not in those transferred to 30% SW. In both groups, the levels declined from 1 to 6 days after transfer although they were statistically significant only in 30% SW on day 3 ($P<0.005$) (Fig. 4). From day 10 onwards muscle glycogen contents were not significantly different in any group.

Experiment 2:

Plasma osmolarity:

Plasma osmolarity were significantly lower after reverse transfer of catfish from 35% SW to TW at all sampling time (Fig. 5).

Plasma glucose:

Reverse transfer of the catfish from 35% SW to TW did not significantly influence plasma glucose profile (Fig. 6).

Liver glycogen:

Liver glycogen content significantly declined ($P< 0.05$) within 1 day after reverse transfer of the catfish from 35% SW to TW (Fig. 7). From day 3 onwards, liver glycogen in both groups were not significantly different.

Muscle Glycogen:

Reverse transfer of catfish to TW showed consistently low muscle glycogen concentration throughout the experimental period (Fig. 8). The levels were significantly low ($P<0.005$) at 24 hr post-reverse transfer.

DISCUSSION

The persistently high plasma osmolarity values in catfish transferred to 30% and 35% SW suggest the lack of a regulative phase in this species which further confirms the observation of Goswami *et al.* (1983) . A similar lack of regulative phase has also been found in another air-breathing catfish, *Clarias batrachus* (Nayyar, 1992) when transferred to higher salinities. The lack of regulative phase though in reverse transfer has also been reported in the barred surfperch, *Amphistichus argenteus*, a marine stenohaline teleost, which shows a continuous decline in plasma osmolarity, plasma sodium and chloride concentration when transferred from 100% SW to dilute SW (Holmes and Lockwood, 1970).

In the present study, when catfish acclimated to 35% SW for 15 days was transferred to TW, there was a significant decline in plasma osmolarity within 3 hr but the fish was able to regain its plasma osmotic pressure similar to TW fish within 24 hr of the reverse transfer. This is understandable since in the absence of any reversal of mechanism in higher salinities in the catfish, the fall in the plasma osmolarity is a consequence of simple decrease of ambient salinity. The initial decrease in plasma osmolarity within 3 hr of transfer to TW suggested that there is a continuous loss of

ions and that fish is not able to immediately reactivate its salt uptake mechanism in TW. Other reports also show decrease in plasma osmolarity of fishes with the decrease in external salinity (Evans, 1980; Woo and Fung, 1981; Mancera, *et al.*, 1993, 1994; Sayer and Reader, 1996).

The results also reveal a significant elevation of plasma glucose within 3 hr of transfer of the catfish both in 30% and 35% SW. It is interesting to note that the magnitude of increase in plasma glucose directly correlates with the magnitude of gradient between plasma osmolarity and the ambient salinity since it is more marked in 35% SW ($P < 0.025$) as compared to 30% SW ($P < 0.05$). A similar pattern was observed in another stenohaline catfish, *Clarias batrachus* (Nayyar, 1992) and also in the euryhaline teleost, *Tilapia mossambica* (Assem and Hanke, 1979a). However, in this catfish, before attaining parity with TW control, plasma glucose profile exhibited a declining pattern both in 30% and 35% SW up to 6 day post-transfer although the levels were significant ($P<0.05$) only at 24 hr in 35% SW and on day 3 in 30% SW. Similar situation was observed during brief aerial emersion in *Limanda limanda* where blood glucose concentration decreased after being hyperglycemic for a short duration (Fletcher, 1984). In stenohaline carp, *Cyprinus carpio*, plasma glucose levels remained significantly

elevated up to 30 days when it was transferred from FW to 1.5% salt water (about 45% SW) (Hegab and Hanke, 1984).

The present study shows that there was no significant change in plasma glucose concentration during reverse transfer of *H. fossilis* from 35% SW to TW which may suggest that quantum of stress was not as high as that of higher salinity. This is also corroborated by the fact that plasma osmotic pressure returns to normal FW levels soon after reverse transfer to FW as against the constantly elevated levels following transfer to higher salinity (See Figs. 1 and 5). Reverse transfer of the stenohaline fish, *Cyprinus carpio* from 1.5% salt water (about 45% SW) to FW also resulted in regulated plasma glucose levels except an initial increase at 3 hr (Hegab and Hanke, 1984). However, other studies show a significantly high blood glucose concentrations during transfer of the fishes to lower salinities (Roche *et al.*, 1989; Woo and Chung, 1995).

Our data show that change in ambient salinity is accompanied by a significant initial decline in liver glycogen content both during transfer of *H. fossilis* to higher salinities and its reverse transfer to TW. It appears that during transfer to higher salinities the catfish actively osmoregulates during the initial phase which is evident from no appreciable increase in the plasma

osmolarity. During this period, any increase in metabolic requirement may possibly be met through liver glycogenolysis. Mobilization of liver glycogen was also observed when the stenohaline fish, *Cyprinus carpio* (Hanke, 1991) and *Clarias batrachus* (Nayyar, 1992) were transferred to higher salinities. Glycogenolysis has also been observed in rainbow trout, *Salmo gairdneri* (Soengas, *et al.*, 1993, 1995a), tilapia, *Oreochromis mossambicus* (Peters) (Hanke, 1991), red sea bream, *Chryophyrus major* L. (Woo and Murat, 1981), Japanese eel, *Anguilla japonica* (So and Chan 1985). Moreover, during later stages of parr-smolt transformation catabolic processes including glycogenolysis predominate (Sheridan *et al.*, 1985). There was also a decrease in liver glycogen content during parr-smolt transformation in Atlantic and coho salmon (Virtanen, 1987; Mayer *et al.*, 1994). In the present study a significant increase in plasma osmolarity beyond 24 hr suggests that the active osmoregulatory control breaks down and the survival in higher salinities is achieved by passive tissue tolerance (Parwez *et al.*, 1983). An apparent lack of any significant change in liver glycogen content beyond 24 hr (except on day 6) supports the above assumption. Similarly during reverse transfer of the catfish from 35% SW to TW, it was able to reactivate its salt uptake mechanism within 24 hr during which plasma osmolarity became more or

less similar to that of TW fish. The energy required to reactivate salt uptake mechanism during reverse transfer may possibly be supplied through liver glycogen as is evident from significant decline in liver glycogen content during the initial phase of 24 hr. The liver glycogen also decreased when the euryhaline teleost, *Tilapia mossambica* was transferred to FW after acclimation for 14 days in 2.5% of salt water (Assem and Hanke, 1979a). Similarly, liver glycogen declined when the red sea bream, *Clarias major* (Woo and Fung, 1981) and *Mylio macrocephalus* (Woo and Wu, 1982) were transferred to reduced salinities. However, it must be mentioned that in the present study plasma glucose profile did not show any clear-cut correlation with liver glycogen content suggesting the possible interplay of other factor(s). Further studies are warranted to elucidate this aspect.

The present results also demonstrate a declining muscle glycogen profile from 24 hr to 6 days following transfer of *H. fossilis* to higher salinities though the levels were significantly low only on day 3 in 30% SW. The significant increase in muscle glycogen levels in 35% SW at 3 hr post-transfer was surprising and not clearly understood. Reverse transfer of 35% SW adapted fish to TW also showed significant reduction at 24 hr post-transfer and the parity

with control group is reached beyond that. A concomitant decline in liver and muscle glycogen was also observed in euryhaline teleost, *Tilapia mossambica*, when transferred to higher salinities (Bashamohideen and Parvatheswararao, 1972). Our data show that salinity induced a significant and an early decline in glycogen in liver as compared to muscle, suggesting that liver glycogen is the immediate source of energy during adaptation of this species of catfish to different salinities.

The present study, therefore, suggests that ambient salinity has discernible effect on carbohydrate metabolism as evidenced from the changes in the profile of plasma glucose and liver and muscle glycogen contents.

Fig. 1. Changes in plasma osmolarity of the catfish, *Heteropneustes fossilis* following transfer from tap water (TW) to 30% and 35% sea water (SW). Asterisks denote significant difference in values compared to control group.

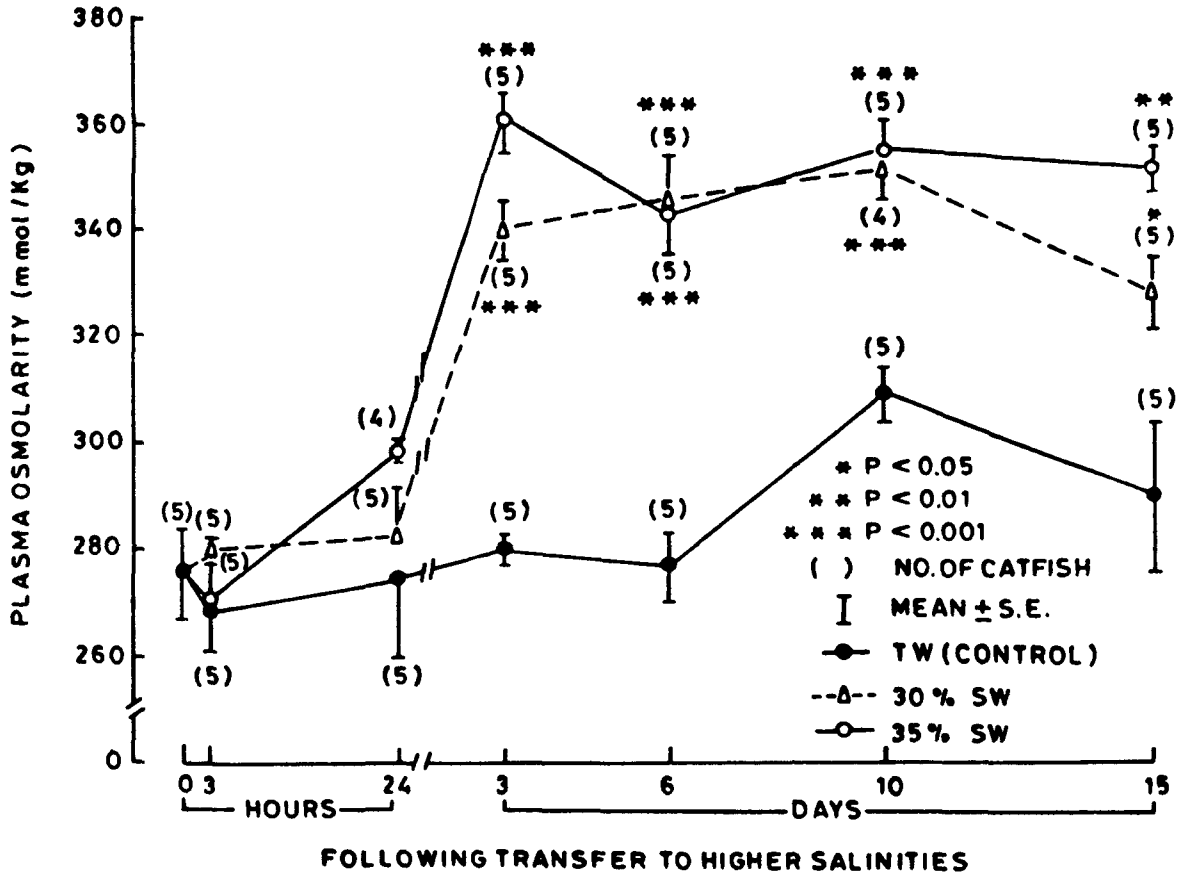
PLASMA OSMOLARITY (m mol/Kg)
380
360
340
320
300
280
260
0
0 3
24
3
6
10
15
HOURS
DAYS
FOLLOWING TRANSFER TO HIGHER SALINITIES
* P < 0.05
** P < 0.01
*** P < 0.001
( ) NO. OF CATFISH
MEAN ± S.E.
TW (CONTROL)
30 % SW
35 % SW

Fig. 1

Fig. 2. Changes in plasma glucose concentration of the catfish, *Heteropneustes fossilis* following transfer from tap water (TW) to 30% and 35% sea water (SW). Asterisks denote significant difference in values compared to control group.

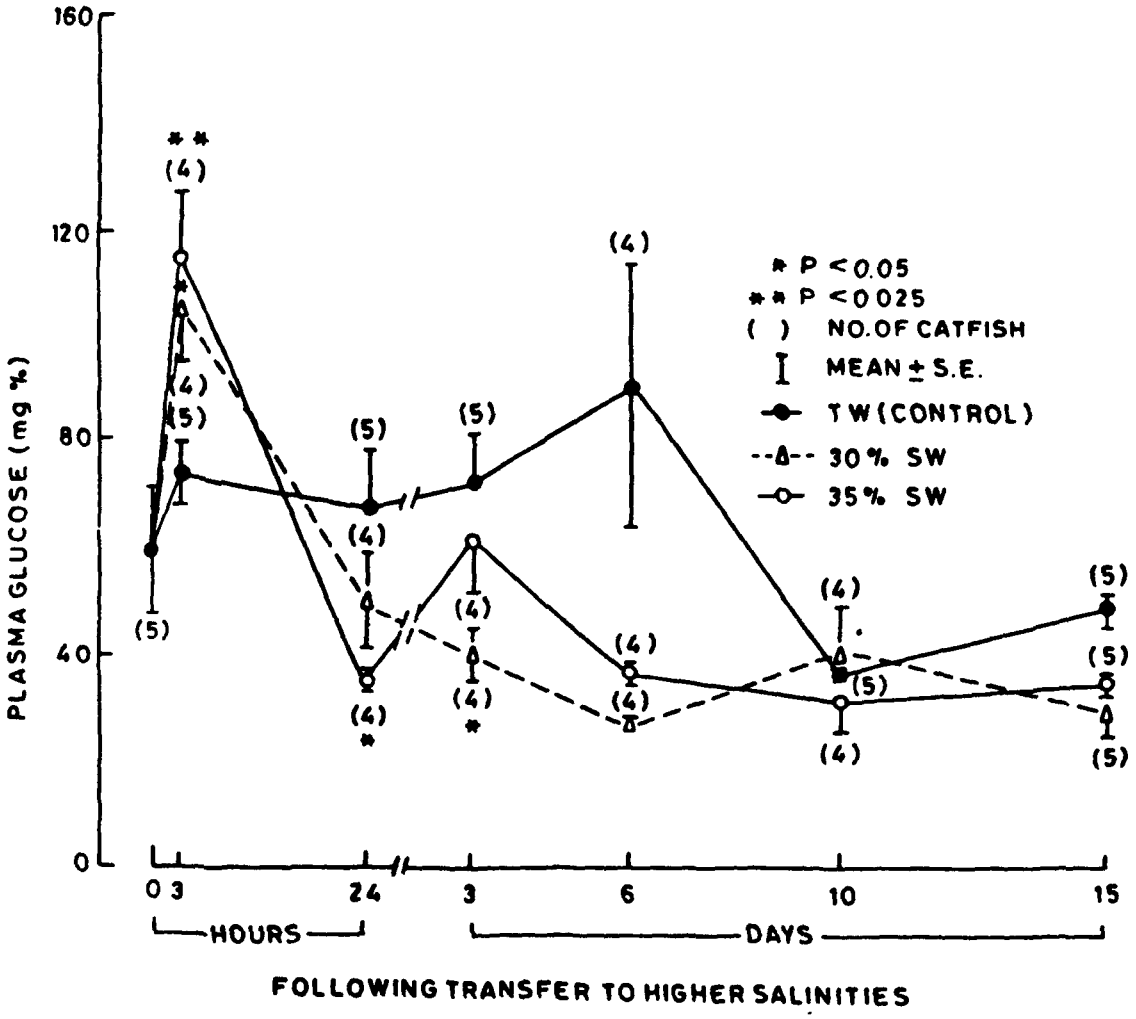
160
120
80
40
0
PLASMA GLUCOSE (mg %)
* P < 0.05
** P < 0.025
( ) NO. OF CATFISH
MEAN ± S.E.
TW (CONTROL)
30 % SW
35 % SW
0 3
24
3
6
10
15
HOURS
DAYS
FOLLOWING TRANSFER TO HIGHER SALINITIES

Fig. 2

Fig. 3. Changes in liver glycogen concentration of the catfish, *Heteropneustes fossilis* following transfer from tap water (TW) to 30% and 35% sea water (SW). Asterisks denote significant difference in values compared to control group.

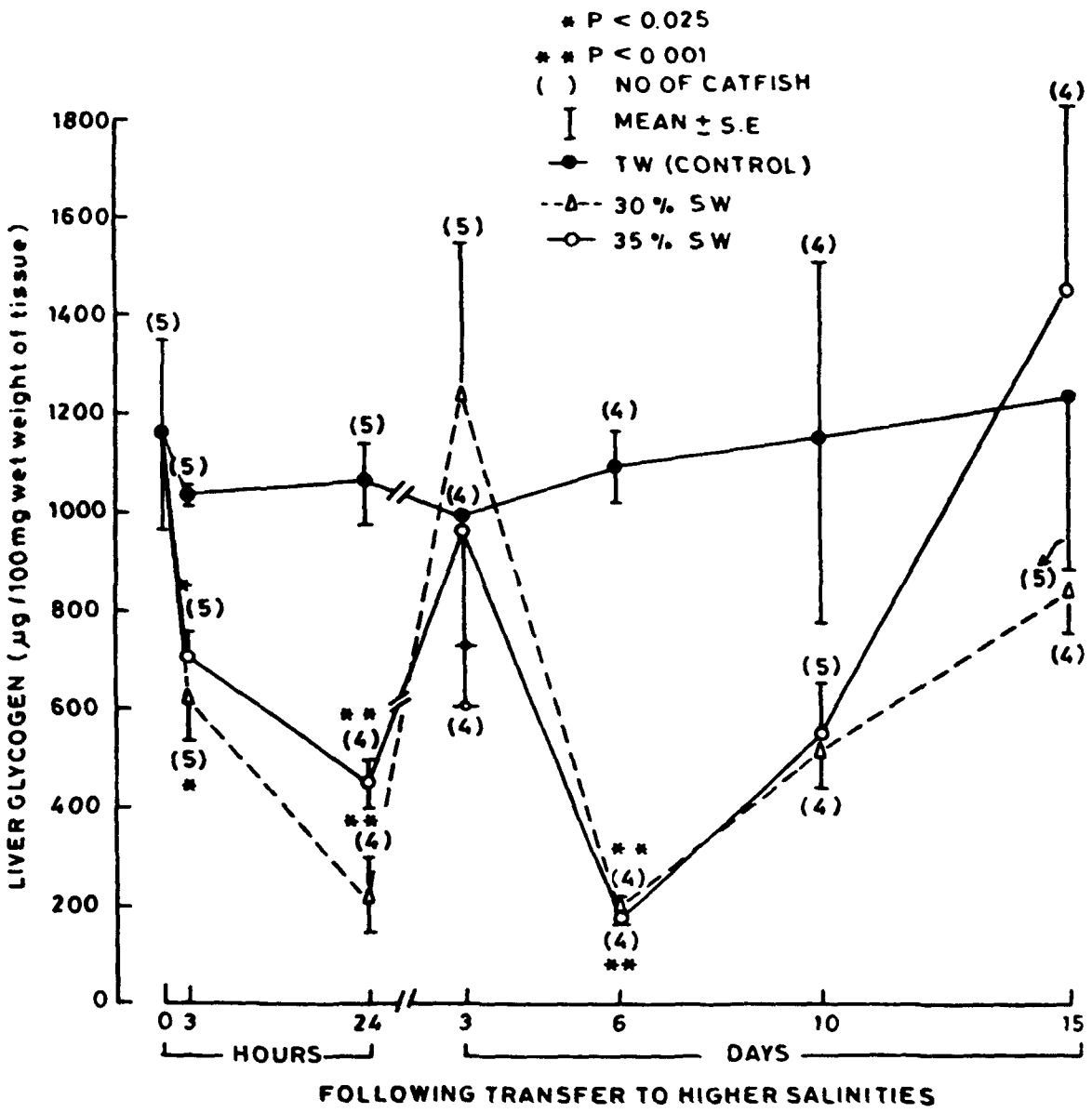
* P < 0.025
** P < 0.001
( ) NO OF CATFISH
MEAN ± S.E
TW (CONTROL)
30 % SW
35 % SW
LIVER GLYCOGEN (µg/100mg wet weight of tissue)
1800
1600
1400
1200
1000
800
600
400
200
0
0 3 24 3 6 10 15
HOURS
DAYS
FOLLOWING TRANSFER TO HIGHER SALINITIES

Fig. 3

Fig. 4. Changes in muscle glycogen concentration of the catfish, *Heteropneustes fossilis* following transfer from tap water (TW) to 30% and 35% sea water (SW). Asterisks denote significant difference in values compared to control group.

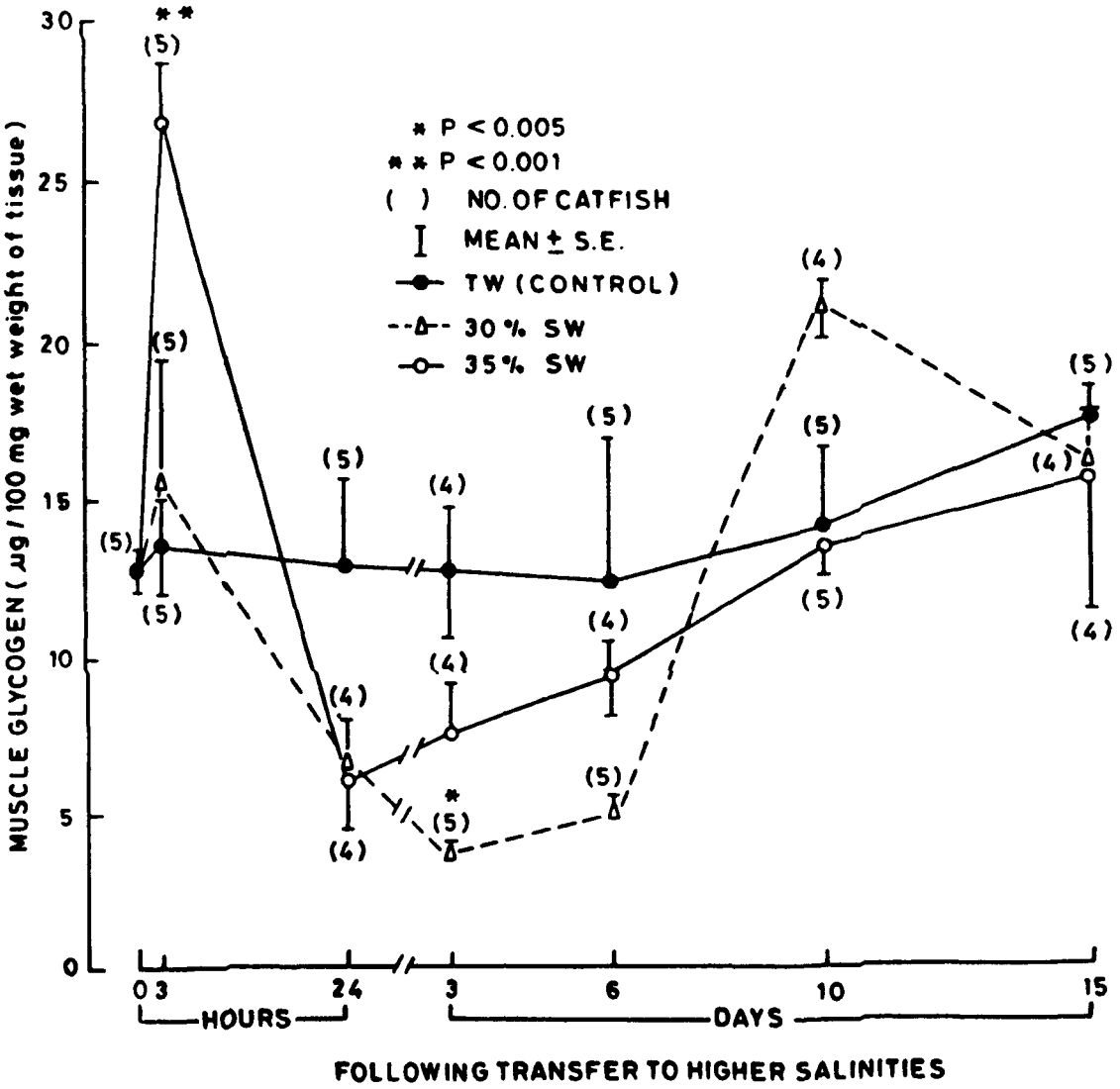
MUSCLE GLYCOGEN (µg / 100 mg wet weight of tissue)
30
25
20
15
10
5
0
* P < 0.005
** P < 0.001
( ) NO. OF CATFISH
MEAN ± S.E.
TW (CONTROL)
30 % SW
35 % SW
0 3
24
3
6
10
15
HOURS
DAYS
FOLLOWING TRANSFER TO HIGHER SALINITIES

Fig.4

Fig. 5. Changes in plasma osmolarity of the catfish, *Heteropneustes fossilis* following reverse transfer to tap water (TW) after acclimation in 35% sea water (SW) for 15 days. Asterisks denote significant difference in values compared to control group.

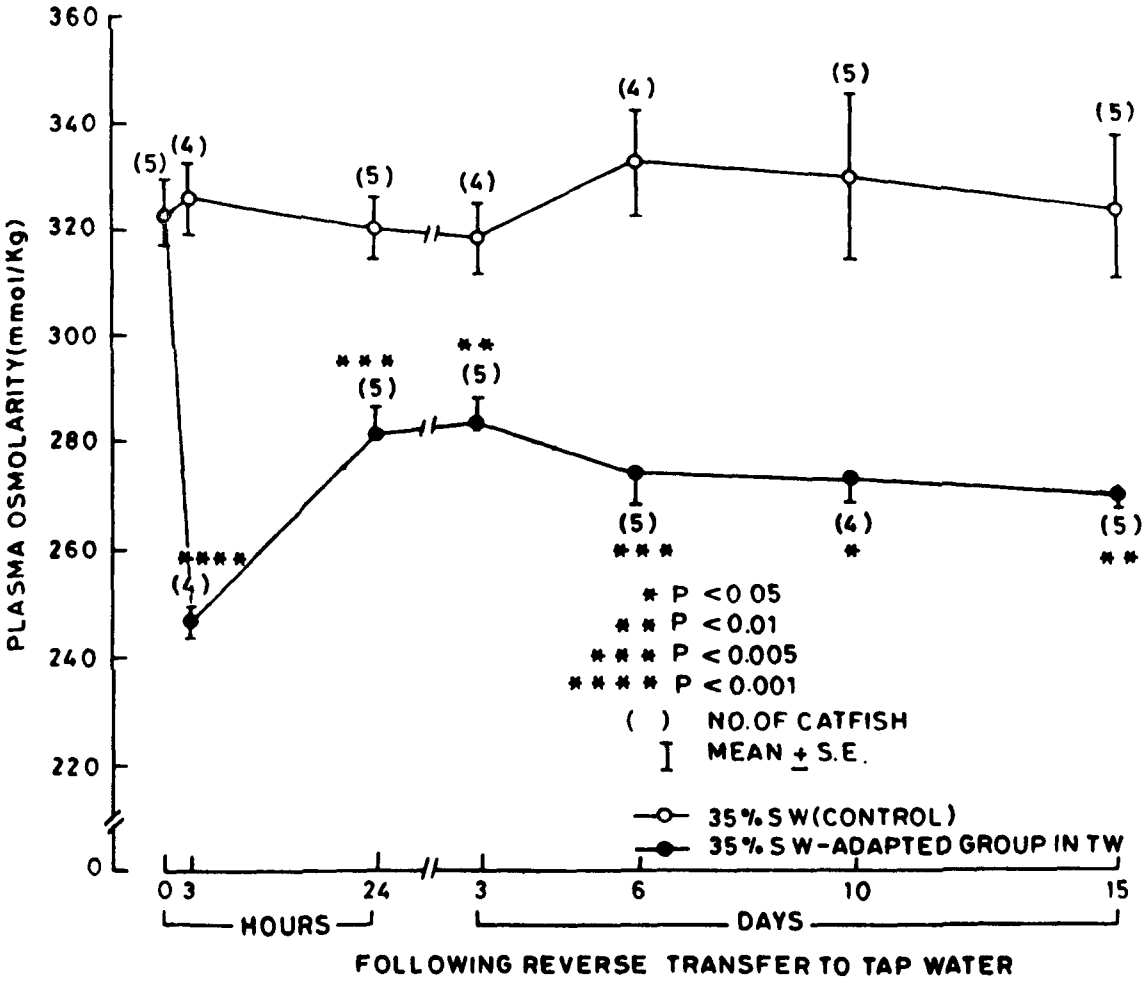
360
340
320
300
280
260
240
220
0
PLASMA OSMOLARITY(mmol/Kg)
(5)
(4)
(5)
(4)
(4)
(5)
(5)
(5)
(5)
(4)
(5)
(4)
(5)
* P <0 05
** P <0.01
*** P <0.005
**** P <0.001
( ) NO.OF CATFISH
I MEAN ± S.E.
35% S W(CONTROL)
35% S W-ADAPTED GROUP IN TW
0 3
24
3
6
10
15
HOURS
DAYS
FOLLOWING REVERSE TRANSFER TO TAP WATER

Fig.5

Fig. 6. Changes in plasma glucose concentration of the catfish, *Heteropneustes fossilis* following reverse transfer to tap water (TW) after acclimation in 35% sea water (SW) for 15 days.

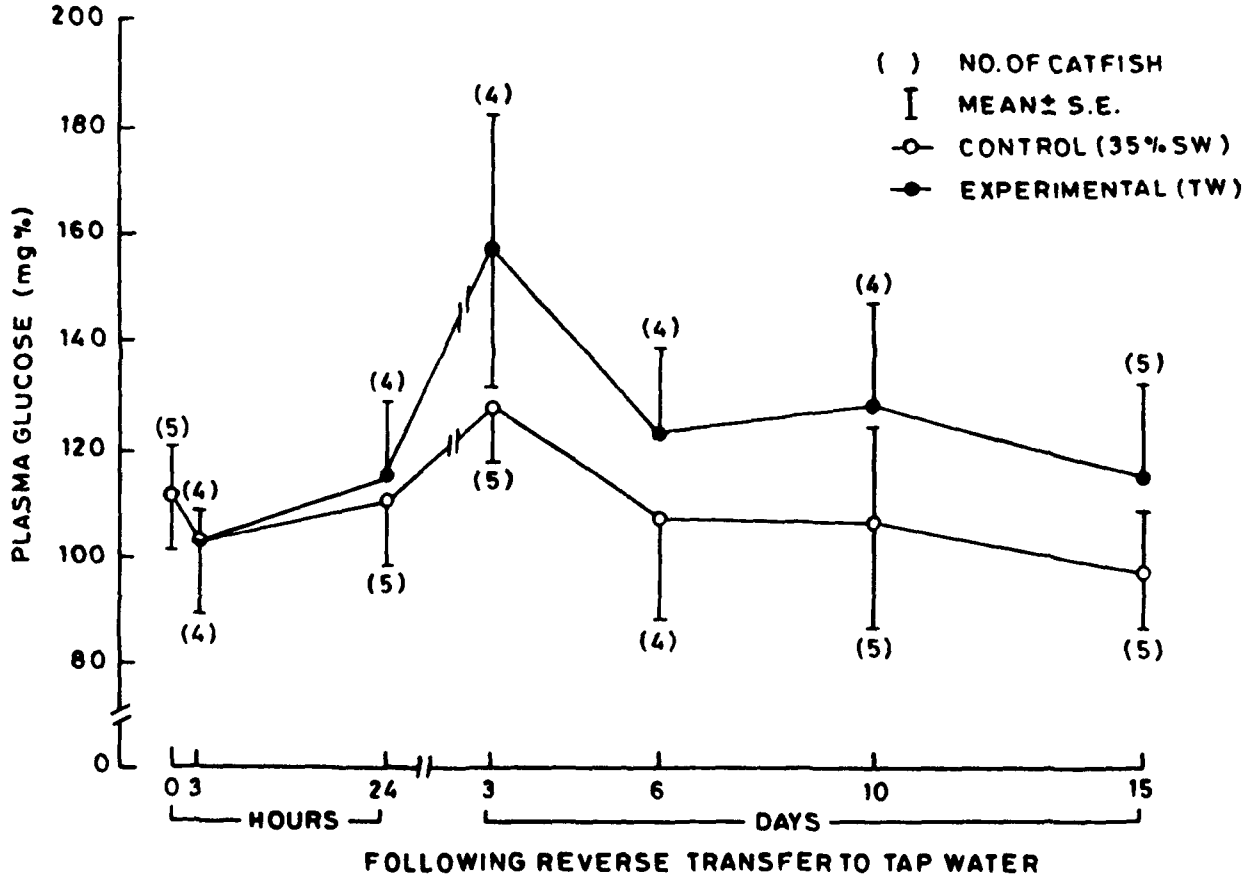
200
180
160
140
120
100
80
0
PLASMA GLUCOSE (mg%)
( ) NO. OF CATFISH
MEAN± S.E.
CONTROL (35% SW)
EXPERIMENTAL (TW)
(5)
(4)
(4)
(4)
(4)
(5)
(4)
(5)
(4)
(5)
(4)
(5)
(5)
0 3
24
3
6
10
15
HOURS
DAYS
FOLLOWING REVERSE TRANSFER TO TAP WATER

Fig. 6

Fig. 7. Changes in liver glycogen concentration of the catfish, *Heteropneustes fossilis* following reverse transfer to tap water (TW) after acclimation in 35% sea water (SW) for 15 days. Asterisks denote significant difference in values compared to control group.

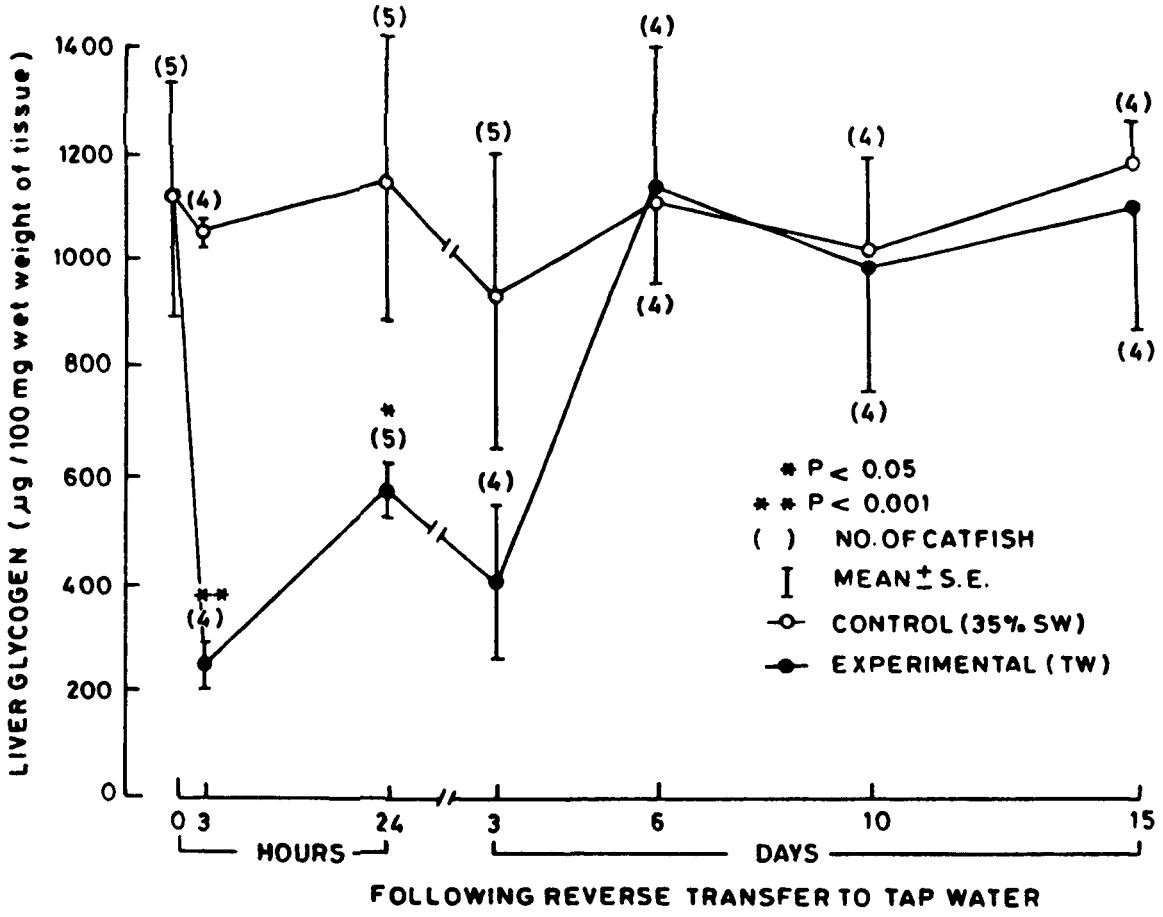
LIVER GLYCOGEN (µg / 100 mg wet weight of tissue)
1400
1200
1000
800
600
400
200
0
(5)
(4)
(5)
(5)
(4)
(4)
(4)
(4)
(5)
(4)
(4)
(4)
*
**
* P < 0.05
** P < 0.001
( ) NO. OF CATFISH
MEAN ± S.E.
CONTROL (35% SW)
EXPERIMENTAL (TW)
0 3
24
3
6
10
15
HOURS
DAYS
FOLLOWING REVERSE TRANSFER TO TAP WATER

Fig. 7

Fig. 8. Changes in muscle glycogen concentration of the catfish, *Heteropneustes fossilis* following reverse transfer to tap water (TW) after acclimation in 35% sea water (SW) for 15 days. Asterisk denotes significant difference in values compared to control group.

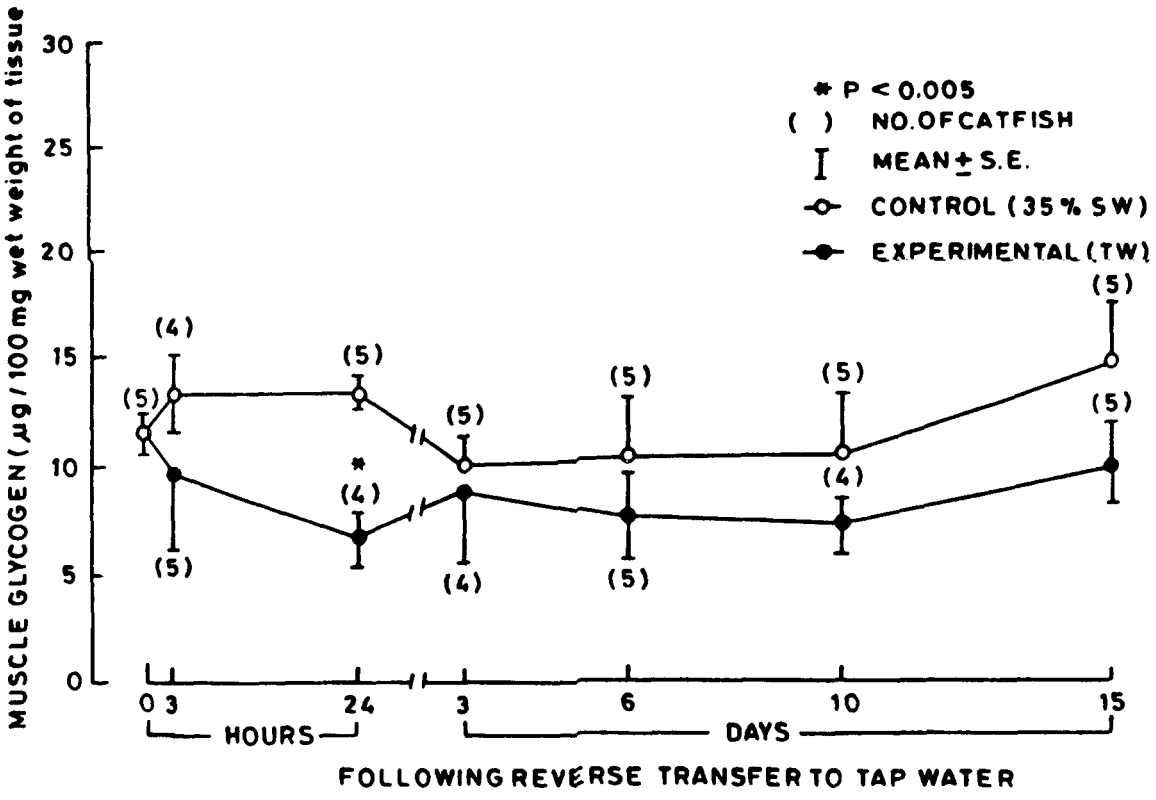
MUSCLE GLYCOGEN (μg / 100 mg wet weight of tissue
30
25
20
15
10
5
0
* P < 0.005
( ) NO. OF CATFISH
MEAN ± S.E.
CONTROL (35 % SW)
EXPERIMENTAL (TW)
(4)
(5)
(5)
(5)
(5)
(5)
(5)
(5)
(4)
(4)
(5)
(5)
(4)
*
0 3
24
3
6
10
15
HOURS
DAYS
FOLLOWING REVERSE TRANSFER TO TAP WATER

Fig.8

CHAPTER III

CHANGES IN THE ACTIVITY PROFILE OF SODIUM/POTASSIUM DEPENDENT ADENOSINE TRIPHOSPHATASE (Na^+/K^+-ATPase) AND SUCCINIC DEHYDROGENASE (SDH) AND CORTISOL AND THYROXINE DURING OSMOTIC ADJUSTMENTS OF THE CATFISH, *HETEROPNEUSTES FOSSILIS*

INTRODUCTION

Osmoregulation in teleost fish is mainly due to the integrated transport activities of gills, gut and the renal system. The basic epithelial structure of these tissues is adapted to provide the appropriate transport mechanisms depending upon whether the fish is in fresh water (FW) or sea water (SW). In the case of FW teleosts, there is an active uptake of salt from the medium into the blood through the branchial apparatus to supplement the diffusional loss of ions coupled with the active reabsorption of ions at renal level. On the other hand, in SW adapted teleosts, there is an excretion of ions against the concentration gradient via gill epithelium and the intestine to offset the diffusional influx of salts.

There is increasing evidence that sodium exchange pump, responsible for sodium ion excretion on one hand and uptake

on the other, is dependent on the energy derived from ATP hydrolysis and would, therefore, be related to Na^+/K^+-ATPase (also known as sodium pump) activity which is inhibited by glycoside such as ouabain. This enzyme is located in the epithelium of osmoregulatory organs such as the gills, kidney and intestine and is involved in the maintenance of extracellular sodium and intracellular potassium concentration. The specific activity of Na^+/K^+-ATPase in the organs of FW and SW fishes appears to be roughly proportional to the level of sodium transport demanded by the environment and the species (Jampol and Epstein, 1970). In SW teleost, Na^+/K^+-ATPase activity is higher in gills (Kamiya, 1972a) and intestine (Jampol and Epstein, 1970) largely because the turnover rate of sodium ions across the gills and intestine is much higher in marine fish than in FW ones. Similarly, active reabsorption occurring at the renal level results in the higher Na^+/K^+-ATPase activity in the kidney of FW teleosts. Histochemical evidence indicates that Na^+/K^+-ATPase, and succinic dehydrogenase (SDH), a mitochondrial marker enzyme, are localized in chloride cells (Keys and Willmer, 1932). These cells show an increase in size and number which parallels with the high activity of Na^+/K^+-ATPase and SDH in hyperosmotic environment. It is, therefore, clear that Na^+/K^+-ATPase and SDH are intimately linked with sodium transport processes in fishes both in SW

as well as in FW and that the acclimation of the fish from FW to SW involves the elaboration not only of Na^+/K^+-ATPase but also the energy producing apparatus to drive this pump expressed biochemically as SDH. Even though the basic osmoregulatory mechanism of the Indian catfish, *H. fossilis* has been worked out, the role of Na^+/K^+-ATPase and SDH has still not been elucidated.

It is well established that various aspects of hydromineral balance in teleosts are regulated by variations in endocrine activity (Kamiya, 1972b; Parwez *et al.*, 1984; Parwez and Goswami, 1985; Boeuf *et al.*, 1989; Dickhoff, 1992; Madsen and Bern, 1992, 1993; Cornell *et al.*, 1994; Parwez *et al.*, 1994; Veillette *et al.*, 1995). Action of several hormones acting on different organs permits the teleost fish to adapt to their environment. Generally, adaptational capacity is based on multifunctional system and cannot be attributed to a definite effect of only one hormone or one organ.

The hormones affecting hydromineral balance in teleosts can be broadly classified into two categories, fast- and slow-acting hormones (Takei, 1993). Fast- or rapidly-acting hormones include epinephrine, neurohypophysial hormones, angiotensin, urotensin and the atrial natriuretic peptide.

They are known to affect ion-pumps as well as ion and water permeability and may be involved in "fine adjustments" in response to rapid changes in the external or internal environment. In contrast, the slow-acting hormones such as cortisol (F) and thyroxine (T_4) take from few to several days to rectify the major osmoregulatory dysfunctions by bringing about the integration in the functions of osmoregulatory organs to achieve whole animal osmoregulation. These hormones, because of the enormous importance, have been studied extensively.

Interestingly, it has been shown in many instances that F as well as T_4 mediate their action through elaboration of Na^+/K^+-ATPase (Boeuf and Prunet, 1985; Madsen and Naamansen, 1989; Olsen *et al.*, 1993; Cornell *et al.*, 1994; Veillette *et al.*, 1995). However, mediation of F action through increased SDH activity in the gills is reported only in few fish species (Langdon *et al.*, 1984) and virtually no data, to the best of our knowledge, exist to show the relationship between T_4 and SDH.

Therefore, the objectives of the present investigation on the catfish were:

(1) To study the changes in the activity profiles of Na^+/K^+-ATPase and SDH at branchial and renal levels

following changes in external salinities of the catfish.

(2) To elucidate the role of F and T_4 following transfer of the catfish to higher salinities.

(3) To assess the causative relationship, if any, of the F and T_4 with Na^+/K^+-ATPase and SDH during adaptation of the catfish in higher salinities.

MATERIALS AND METHODS

I. Experimental protocol:

The experiment was conducted on well acclimatized catfish under controlled laboratory conditions as described in Chapter I.

The catfish were transferred from tap water (TW) to 30% and 35% SW. TW group served as control. Four to five fish from each group were sampled at 3, 24 hr, 3,6, 10 and 15 days post-transfer. Blood was collected from the caudal artery as described in Chapter I and plasma thus obtained were analysed for F as described in Chapter I and T_4 as detailed later in this chapter. The gills were excised and the kidney was dissected out to estimate the activities of the enzymes, Na^+/K^+-ATPase and SDH according to the methods described

below. SDH activity was determined only at 24 hr, 6, 10 and 15 days post-transfer. The data are presented in Figs. 2-7.

II Enzyme Assay:

Homogenization and centrifugation:

Both the gill arches (approximate weight 0.18 g) and kidneys (approximate weight 0.2 g) were immersed in ice-cold homogenizing medium (HHM) (composition: 0.25 M sucrose, 5 mM disodium EDTA, 0.1% deoxycholate, pH 7.2) in 1:20 ratio *i.e.*, approximately 100 mg tissue in 2 ml of HHM and were homogenized with power driven teflon pestle in glass homogenizer kept in ice jacket. The homogenate was centrifuged at 10,000 rpm for 20 min at 4°C and the supernatant was frozen in liquid nitrogen and kept at -20°C until assayed.

1. Na^+/K^+-ATPase:

This enzyme was assayed following the method of Evans *et al.*, (1973) with minor modifications.

An aliquot of 0.1 ml of supernatant was added to two sets of precooled incubation tubes - one containing 0.5 ml salt solution (400 mM NaCl and 40 mM KCl) and the other 0.5 ml ouabain (2 mM), followed by addition of 0.2 ml double distilled water (DDW). Both sets were preincubated for 2-3 min at 37°C. Then, 0.2 ml of 35 mM ATP-$MgCI_2$ solution (pH

7.0) was added at 30 seconds interval and incubated for 20 min at 37^{o}C in a shaking water bath. The reaction was terminated by the addition of 1.0 ml of 20% trichloroacetic acid (TCA) at 30 seconds interval in the same order as in the addition of ATP. The mixture was vortexed briefly and centrifuged at 10,000 rpm for 15 min at 4^{o}C.

Inorganic phosphate (Pi):

Pi was determined according to the method of Rockstein and Herron (1951). Briefly, to a 0.2 ml of the sample of standard phosphate (0.25 - 10 µmol Pi/o.2 ml) and unknown solution, 9 ml of acid molybdate solution was added (to 200 ml of solution containing 25 ml of 6.6% stock molybdate solution were slowly added 25 ml of 7.5 N H_2SO_4; stable indefinitely at room temperature), followed by 0.8 ml freshly prepared ferrous sulphate solution (to a 50 ml volumetric flask were added 5 g ferrous sulphate hepta hydrate followed by 1 ml 7.5N H_2SO_4 and made to mark with DDW). A similar blank was prepared using 0.2 ml of DDW plus acid molybdate and ferrous sulphate solution as in the standard or unknown preparation. Pi concentration was obtained in µmol Pi/0.2 ml.

Protein:

Protein was estimated by the method of Bailey (1962) which is the modification of Lowry *et al.*, (1951). In this method, to 0.1 to 0.2 ml of sample (containing 10-100 µg of protein) or to bovine serum albumin standard (range 5-100 µg/0.2 ml) was added 1.0 ml of reagent C (prepared by the combination of reagent A: 2% Na_2Co_3 in 0.1N NaOH and reagent B: 0.5% $CuSO_4.5H_2O$ in 1% sodium citrate) and the solution was allowed to stand at room temperature for 10 min. To this, 0.1 ml of reagent D (Follin-Ciocalteu reagent diluted with DDW to give a solution 1N in acid) was pipetted rapidly into the mixture with thorough mixing and the optical density (O.D.) was taken at 750 mµ after a time interval of 0.5 to 2 hr.

The activity of Na^+/K^+ - ATPase was expressed as: µmol Pi/mg protein/hr.

2. SDH:

SDH activity was determined according to the method of Penington (1961) with minor modifications. One hundred µl of the sample volume was raised to 1 ml with assay buffer (pH 7.5) containing 50 mM potassium phosphate, 0.1% INT (2-(p-Iodophenyl)-3-(p-nitrophenyl)-5-phenyltetrazolium chloride), 50 mM sodium succinate and 25 mM sucrose. The tubes were incubated for 15 min in a shaking water bath at 37°C,

reaction was terminated with 1 ml 10% TCA and formazan was extracted with 2.5 ml ethyl acetate. OD was measured at 490 mμ and the tube containing only ethyl acetate served as blank. Protein was estimated as described earlier.

The results are expressed as:

mU formazan/mg protein/hr

III Hormonal Assay:

(1) Plasma F:

Plasma F was assayed according to the method described in Chapter I.

(2) Plasma T_4:

The T_4 content of plasma samples was measured by RIA according to the method of Tagawa and Hirano (1987). For the assay, 10 μl of the plasma samples or T_4 standards was added in duplicate to the assay tubes; then, followed by 250 μl of following mixture (bovine γ-globulin (Cohn fraction II Sigma),150 mg; 0.11 M barbital buffer (pH 8.6) containing 0.1% gelatin, 100 ml; 50 μl of antiserum; and 50 μl of radioactive T_4 (about 20,000 cpm) solution). Antiserum was previously diluted 50 times to give a concentration which would result in the binding of 30-40% labelled T_4 without addition of unlabelled T_4. Tubes were incubated overnight at

4°C. Antibody was then precipitated by addition of 0.5 ml cold 20% (w/w) polyethylene glycol 6000. After incubation for 30 min at 4°C, the tubes were centrifuged at 3000 rpm for 30 min at 4°C. The supernatant was aspirated and the precipitate was counted in a gamma counter. Intraassay and interassay coefficients of variation were 2.9% (n=5) and 5.9% (n=6), respectively at about 40% binding.

Parallelism of T_4:

Serial dilutions of plasma from *H. fossilis* exhibited inhibition curve parallel to the standard curve (Fig. 1)

RESULTS

Plasma F:

Plasma F levels significantly decreased within 3 hr post-transfer in 30% SW ($P<0.05$) and then on day 3 both in 30% ($P<0.005$) and 35% SW ($P<0.001$). The levels thereafter were equivalent to those of TW control (Fig. 2).

Plasma T_4:

Transfer of the catfish to 35% SW resulted in a significantly high levels of plasma T_4 on day 3 ($P<0.01$). However, plasma T_4 titers were always higher throughout the

duration of the experiment both in 30% and 35% SW over that of the TW control (Fig. 3).

Na^+/K^+-ATPase activity in gills:

Gill Na^+/K^+-ATPase activity did not show any significant difference following transfer of the catfish to 30% and 35% SW (Fig. 4).

Na^+/K^+-ATPase activity in kidney:

A significant increase in the activity of the enzyme was observed 1-3 days after transfer of catfish to 30% SW. In those transferred to 35% SW, a significant increase ($P<0.005$) in the enzyme activity was noted only at 3 hr post-transfer. (Fig. 5).

SDH activity in gills:

SDH activity in the gills increased significantly ($P<0.005$) in 35% SW at 24 hr post-transfer and on day 6 both in 30% and 35% SW ($P<0.01$) beyond which it became almost equivalent to TW control (Fig. 6).

SDH activity in kidney:

No significant difference was observed in SDH activity profile in kidney following transfer to 30% and 35% SW (Fig. 7).

DISCUSSION

Since the works of Epstein *et al.* (1967) and Kamiya and Utida (1968) on killifish (*Fundulus* sp.) and eel, *(Anguilla japonica)*, the important role played by Na^+/K^+-ATPase in ion transfer has been confirmed in a number of teleost species (De Renzis and Bornancin, 1984). It has been found that the activity of this enzyme increases significantly at branchial level following transfer of fish to higher salinities or *vice versa* (Hegab and Hanke, 1984; Madsen and Naamansen, 1989; Kültz *et al.*, 1992; Sun *et al.*, 1994) but remains more or less constant in kidney of fishes as in *Anguilla rostrata* (Jampol and Epstein, 1970), *Oncorhynchus mykiss* (Jürss *et al.*, 1985), *Salmo salar* (McCormick *et al.*, 1989) or shows decrease as found in *Dicentrarchus labrax, Crenimugil labrosus* (Lasserre, 1971); *Fundulus heteroclitus* (Epstein *et al.*, 1969); *Morone saxatilis* (Madsen *et al.*, 1994) when transferred from FW to SW.

The present study on the catfish, *H. fossilis* reveals that this fish lacks the classic acclimation model since Na^+/K^+-ATPase activity did not show any significant change in the gill, while in kidney it significantly increased when the catfish were transferred to 30% and 35% SW.

As far as Na^+/K^+- ATPase in the gills is concerned, the

data corroborate the observations on *Cyprinodon salinus* (Stuenkel and Hillyard, 1980), *Gasterosteus aculeatus* (Jürss *et al.*, 1982b), *Platichthys flesus* (Stagg and Shuttleworth, 1982), *Oreochromis mossambicus* (Kültz and Jürss, 1991), *Morone saxatilis* (Madsen *et al.*, 1994). In *Oreochromis mossambicus*, Kültz *et al.* (1992) observed only a slight increase in branchial Na^+/K^+-ATPase between groups in FW and 35‰ but a very strong increase between groups in 35‰ and 45‰. From these observations, they postulated that the increase in Na^+/K^+-ATPase levels in gills starts at environmental salinities greater than 34‰ SW which represents the threshold at which Na^+/K^+-ATPase activity exceeds the FW level. The lack of increase in branchial Na^+/K^+-ATPase in higher salinities supports the idea that catfish gills may not be able to reverse their function of salt absorption in FW to salt excretion in SW (Goswami *et al.*, 1983). In the absence of any salt excretory role of catfish gills in higher salinities, the entire load of excreting extra ions may presumably be falling on the renal route which can handle salt maximally only up to the production of isoosmotic urine due to the absence of the loop of Henle. It is logical to expect that the unexcreted extra salt load may result in an increase in plasma osmotic pressure which is substantiated by the persistent high plasma osmolarity of the catfish both in 30% and 35% SW

(Chapter II, Fig. 1). Such a situation also exists in tilapia (*Oreochromis mossambicus*), which upon transfer, experienced very high plasma osmolarity and died at 6 hr in 30‰ and at 12 hr in 20‰ . The fish in both situations exhibited only a small increase in gill Na^+/K^+-ATPase activity which has been explained as due to insufficient activation of salt-excretory mechanism (Hwang *et al.*, 1989).

The increased Na^+/K^+-ATPase at renal level in the catfish, *H. fossilis* may be attributed to the increased ion secretion by kidney tubule. This assumption is supported by increase in urine osmolarity following transfer of *H. fossilis* to 1.1% NaCl solution (377 mOsm/l) (Goswami *et al.*, 1983). Jampol and Epstein (1970) found relatively high concentration of Na^+/K^+-ATPase in the aglomerular kidney of the goosefish, *Lophius americanus* which is an inhabitant of SW. Obviously, the activity of the enzyme cannot in this case be correlated with reabsorption of sodium from a glomerular filtrate. According to these authors, Na^+/K^+-ATPase is involved in the process of secretion of divalent ions and organic bases into the urine.

The increase in SDH profile of gills following transfer of *H. fossilis* to higher salinities is in good agreement with the previous studies and points towards an elevated metabolic

requirement for adaptation in higher salinities (Doneen, 1981). There are only few reports on branchial SDH in euryhaline teleosts which mostly show increased activity after SW acclimation such as in *Anguilla anguilla* (Sargent *et al.*, (1975), *Gillichthys mirabilis* (Doneen, 1981), *Salmo salar* (Langdon *et al.*, 1984) and *Morone saxatilis* (Madsen *et al.*, 1994). Nevertheless, Epstein, *et al.*, (1967) and Conte (1969) did not find a significant difference between SDH activity of the gills of FW and SW fish. Species differences and methodological variations in biochemical assay of SDH are the suggested reasons for such discrepancies (Sargent *et al.*, 1975). However, to the best of our knowledge there is no report on the changes in SDH activity profile of kidney in fishes transferred to higher salinities.

There are several studies reporting hormonal control of osmoregulation in teleost fishes. Of the vast array of hormones, F and T_4 are considered to be the principal hormones concerned with hydromineral balance in higher salinities (Young, 1986; Dange, 1986; Madsen, 1990a, b; Madsen *et al.*, 1994).

There is increasing evidence that plasma F levels in teleost exhibit a significant increase following transfer of fish to higher salinities (Assem and Hanke, 1981; Hegab and Hanke, 1984; Nichols and Weisbart, 1985; Madsen *et al.*,

1994). However, there are also contradicting observations. For instance, a transitory increase in plasma F was observed after the transfer from SW to brackish water in *Anguilla anguilla* (Leloup-Hatey, 1974); *Chrysophrys major* (Ishiota, 1980); *Sarotherodon mossambicus* (Assem and Hanke, 1981) and *Fundulus heteroclitus* (Jacob and Taylor, 1983). Higher levels of F were also recorded in brackish water with respect to SW in *Mugil cephalus* and *Platichthys stellatus* (Johnson, 1973); *Dicentrarchus labrax* (Roche *et al.*, 1989) and *Sparus aurata* (Mancera *et al.*, 1993). In the present study, a significant decline in plasma F levels was observed both in 30% and 35% SW after 3 days of transfer. The decreased plasma F levels in higher salinities may not be due to the reduced production but rather to enhanced utilization and clearance (Hirano *et al.*, 1976; Goswami *et al.*, 1983; Nayyar, 1992). In general, this hormones is thought to promote excretion of electrolytes in fish living in hyperosmotic media by stimulating the ion transporting tissues such as gills, kidney and intestine (Pickford *et al.*, 1970; Dange, 1986). It seems likely that this hormone may be mediating its action through elaboration of Na^+/K^+-ATPase at renal level, since the activity of this enzyme exhibits a significant increase in kidney following transfer of the catfish to higher salinities. However, lack of increase in

branchial Na^+/K^+-ATPase following catfish transfer to higher salinities appears enigmatic. One possibility is that the enhancement of branchial Na^+/K^+-ATPase may require higher titers of plasma F and endogenous F levels may well be below required threshold. This assumption is further strengthened by the fact that the exogenous administration of FA into SW transferred catfish resulted in significant increase in the activity of gill Na^+/K^+-ATPase at 24 hr (Chapter IV, Fig. 4).

There are evidence that when fishes are transferred from FW to SW, plasma T_4 levels may increase (Folmar and Dickhoff, 1981; Redding *et al.*, 1984; Shivakumar and Jayaraman, 1984; Leloup and De Luze, 1985; Woo and Chung, 1995) or decrease (Grau, *et al.*, 1980; Specker, 1980; Dickhoff *et al.*, 1982; Boeuf *et al.*, 1989; Marc *et al.*, 1995) or may remain unchanged (Milne and Leatherland, 1980, Boeuf and Le Bail, 1990). In the present study, except on day 3 in 35% SW, there was no significant change in plasma T_4 levels when the catfish were transferred to 30% and 35% SW. However, plasma T_4 levels were consistently higher both in 30% and 35% SW throughout the duration of experiment.

Thus, it may be concluded from the foregoing discussion that the catfish gills may not be able to reverse their function from salt uptake in FW to salt excretion in SW and that elimination of monovalent as well as divalent ions is

being performed by the kidney. Contrary to the classical model, this function is not being shared by the gills, as evident from insignificant changes in branchial Na^+/K^+-ATPase activities and significantly increased activity of this enzyme at renal level in higher salinities. However, role of T_4 during SW transfer of the catfish is not clear.

Fig. 1. Standard curve for thyroxine showing parallelism with serial dilutions of plasma from catfish. B/Bo is per cent relative binding. Each point represents the average of duplicate determinations.

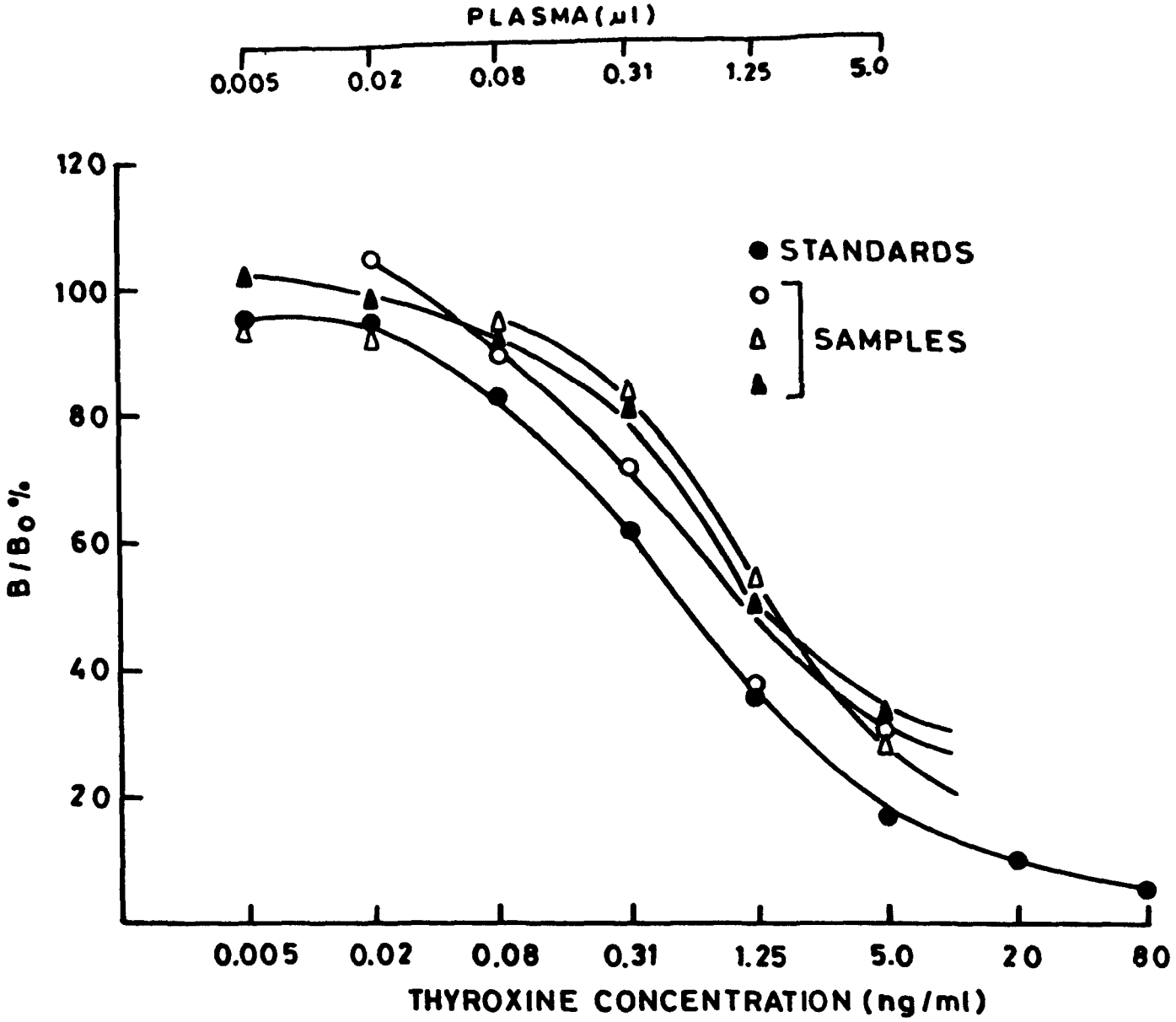
PLASMA (µl)
0.005
0.02
0.08
0.31
1.25
5.0
120
100
80
60
40
20
B/Bo %
STANDARDS
SAMPLES
0.005
0.02
0.08
0.31
1.25
5.0
20
80
THYROXINE CONCENTRATION (ng/ml)

Fig. 1

Fig. 2. Changes in plasma cortisol of the catfish, *Heteropneustes fossilis* following transfer from tap water (TW) to 30% and 35% sea water (SW). Asterisks denote significant difference in values compared to control group.

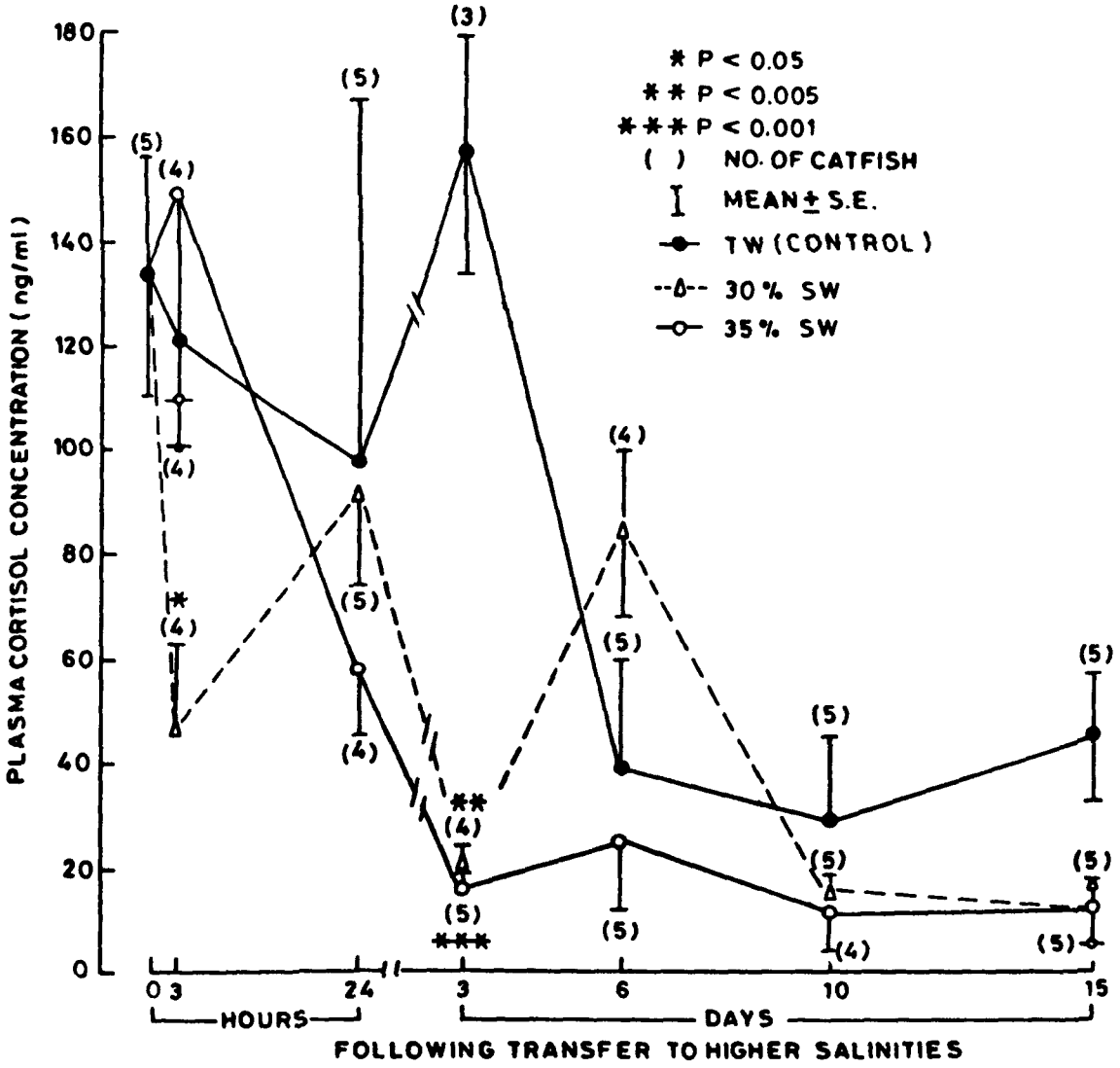
* P < 0.05
** P < 0.005
*** P < 0.001
( ) NO. OF CATFISH
MEAN ± S.E.
TW (CONTROL)
30 % SW
35 % SW
PLASMA CORTISOL CONCENTRATION (ng/ml)
0
20
40
60
80
100
120
140
160
180
0 3
24
3
6
10
15
HOURS
DAYS
FOLLOWING TRANSFER TO HIGHER SALINITIES

Fig.2

Fig. 3. Changes in plasma thyroxine of the catfish, *Heteropneustes fossilis* following transfer from tap water (TW) to 30% and 35% sea water (SW). Asterisk denotes significant difference in values compared to control group.

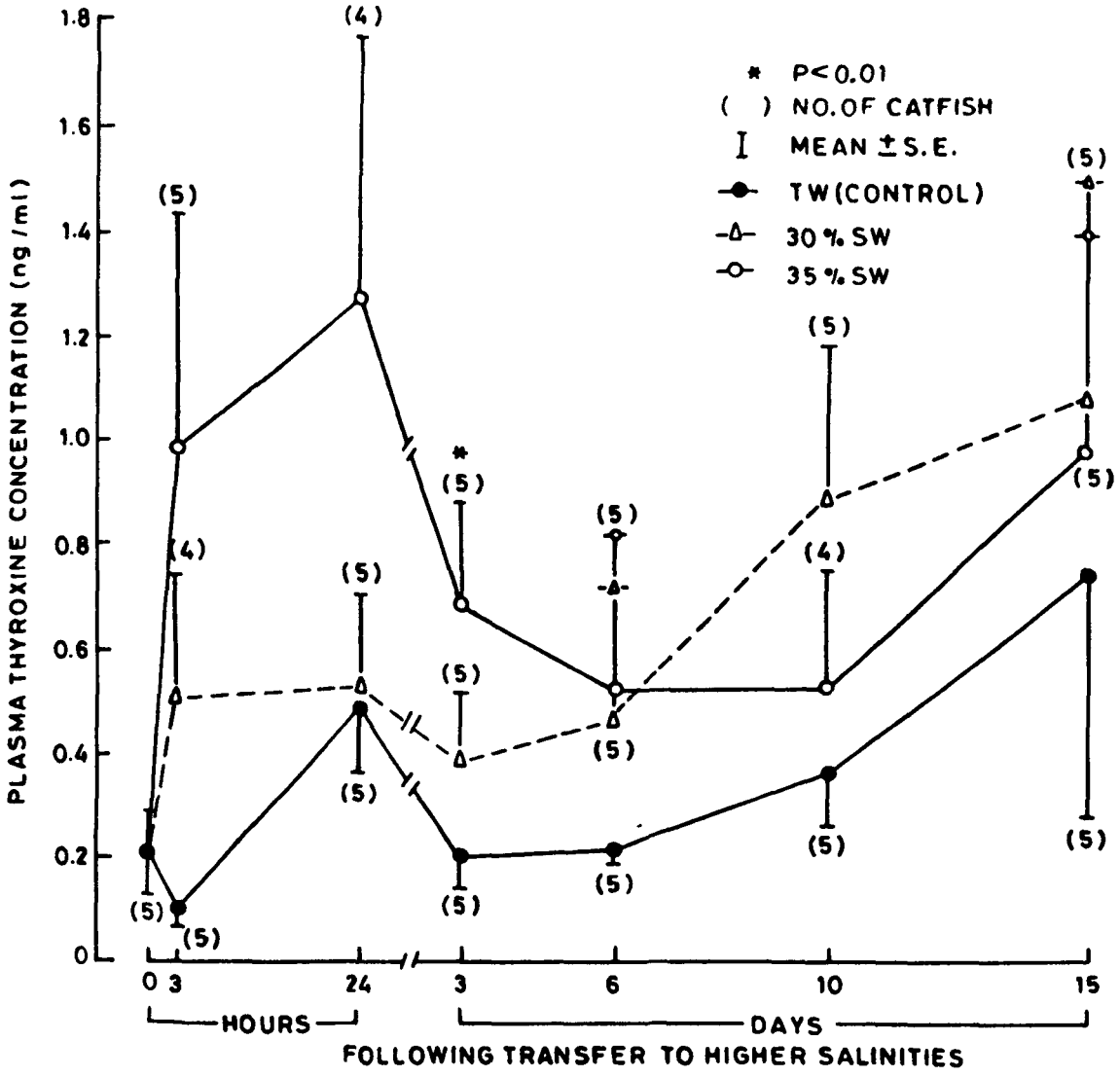
* P<0.01
( ) NO. OF CATFISH
I MEAN ± S.E.
TW (CONTROL)
30 % SW
35 % SW
PLASMA THYROXINE CONCENTRATION (ng/ml)
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
0 3 24 3 6 10 15
HOURS
DAYS
FOLLOWING TRANSFER TO HIGHER SALINITIES

Fig. 3

Fig. 4. Changes in gill sodium/potassium dependent adenosine triphosphatase (Na^+/K^+-ATPase) activity of the catfish, *Heteropneustes fossilis* following transfer from tap water (TW) to 30% and 35% sea water (SW).

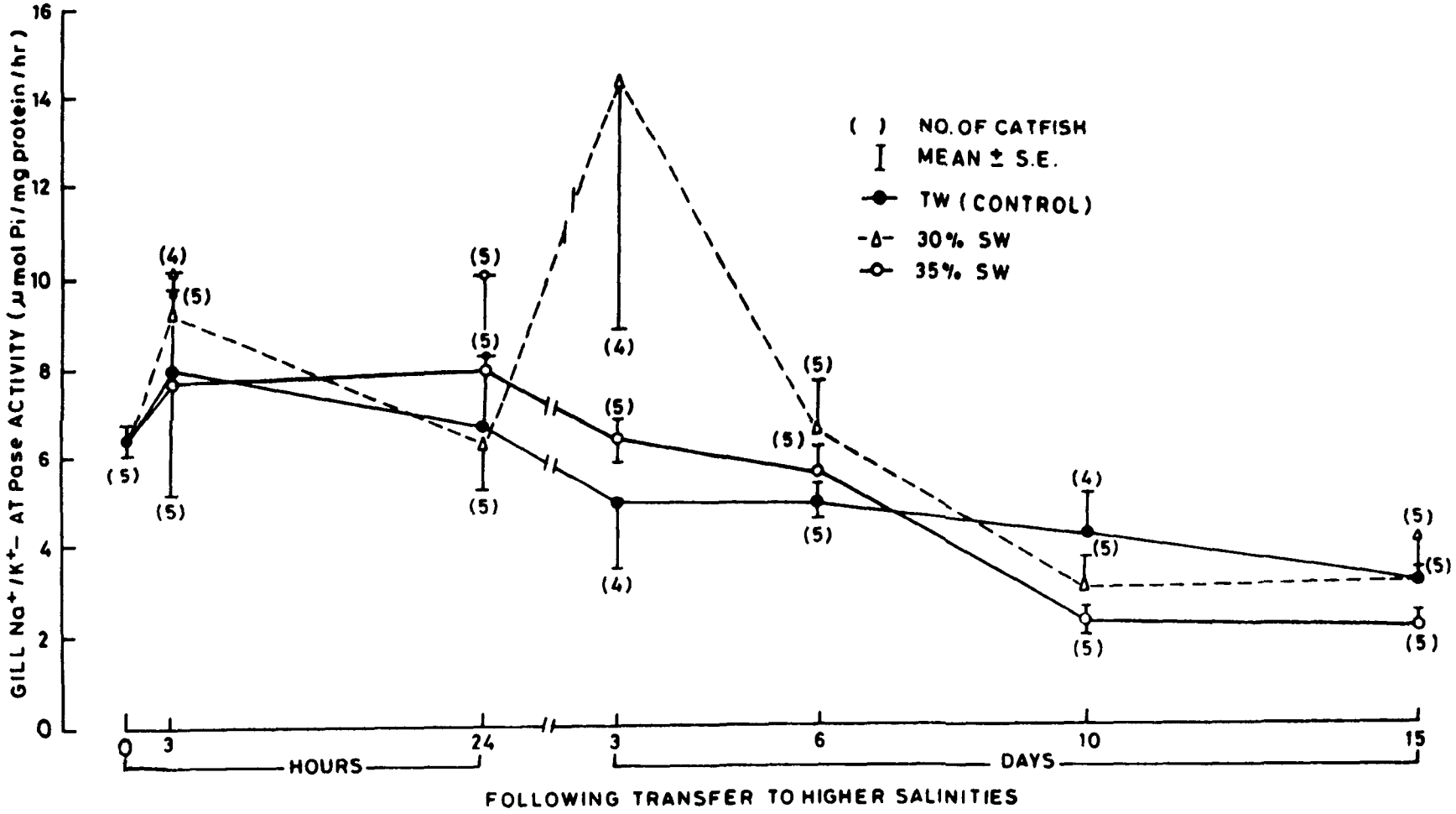
GILL Na+/K+- AT Pase ACTIVITY (μmol Pi/mg protein/hr)
16
14
12
10
8
6
4
2
0
( ) NO. OF CATFISH
MEAN ± S.E.
TW (CONTROL)
30% SW
35% SW
(4)
(5)
(5)
(5)
(5)
(4)
(5)
(5)
(5)
(4)
(5)
(5)
(5)
(5)
(5)
(5)
(5)
(5)
0
3
24
3
6
10
15
HOURS
DAYS
FOLLOWING TRANSFER TO HIGHER SALINITIES

Fig.4

Fig. 5. Changes in kidney sodium/potassium dependent adenosine triphosphatase (Na^+/K^+-ATPase) activity of the catfish, *Heteropneustes fossilis* following transfer from tap water (TW) to 30% and 35% sea water (SW). Asterisks denote significant difference in values compared to control group.

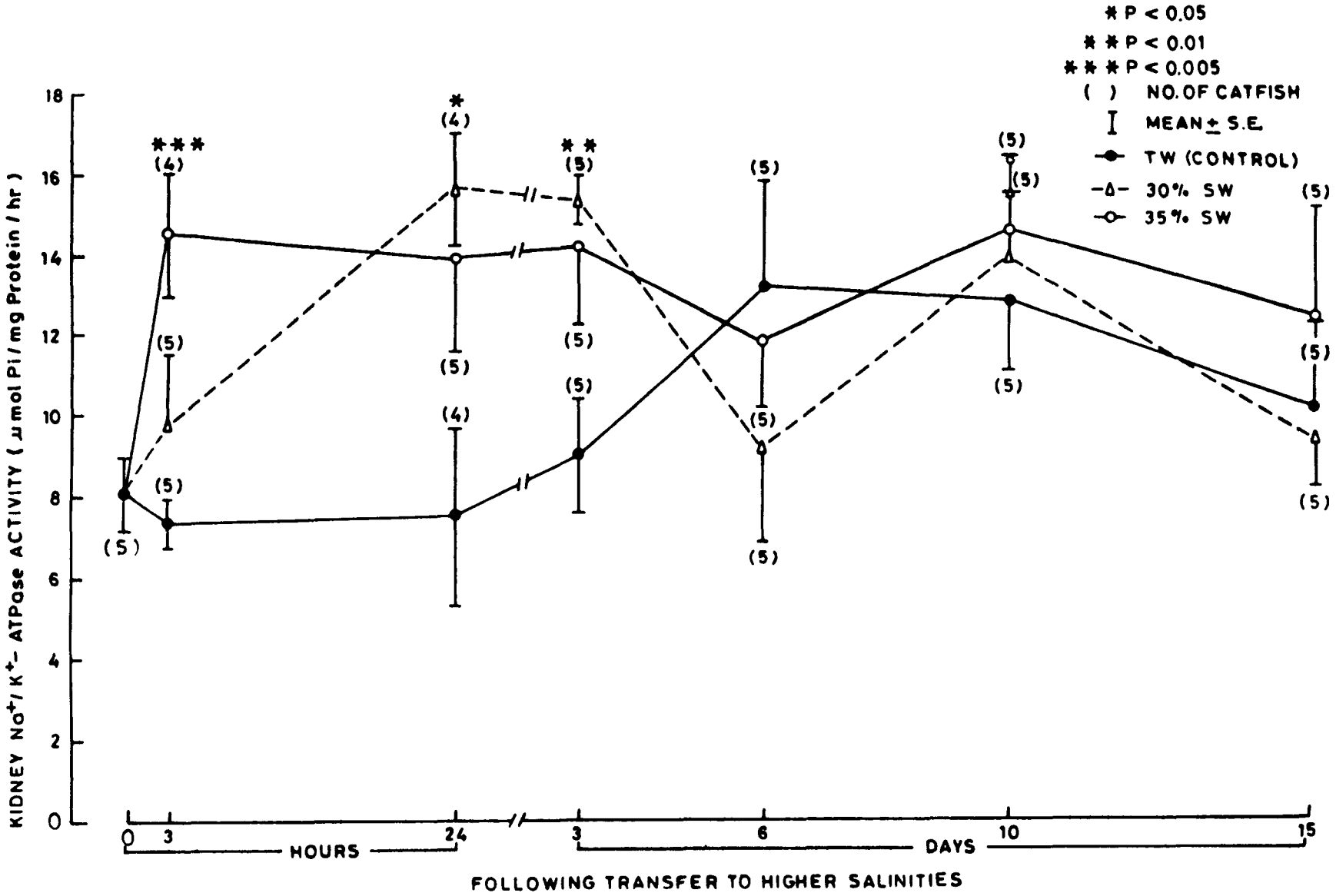
* P < 0.05
** P < 0.01
*** P < 0.005
( ) NO. OF CATFISH
I MEAN ± S.E.
TW (CONTROL)
30% SW
35% SW
KIDNEY No^+/K^+- ATPase ACTIVITY (μ mol Pi / mg Protein / hr)
0
2
4
6
8
10
12
14
16
18
HOURS
DAYS
0
3
24
3
6
10
15
FOLLOWING TRANSFER TO HIGHER SALINITIES

Fig.5

Fig. 6. Changes in gill succinic dehydrogenase (SDH) activity of the catfish, *Heteropneustes fossilis* following transfer from tap water (TW) to 30% and 35% sea water (SW). Asterisks denote significant difference in values compared to control group.

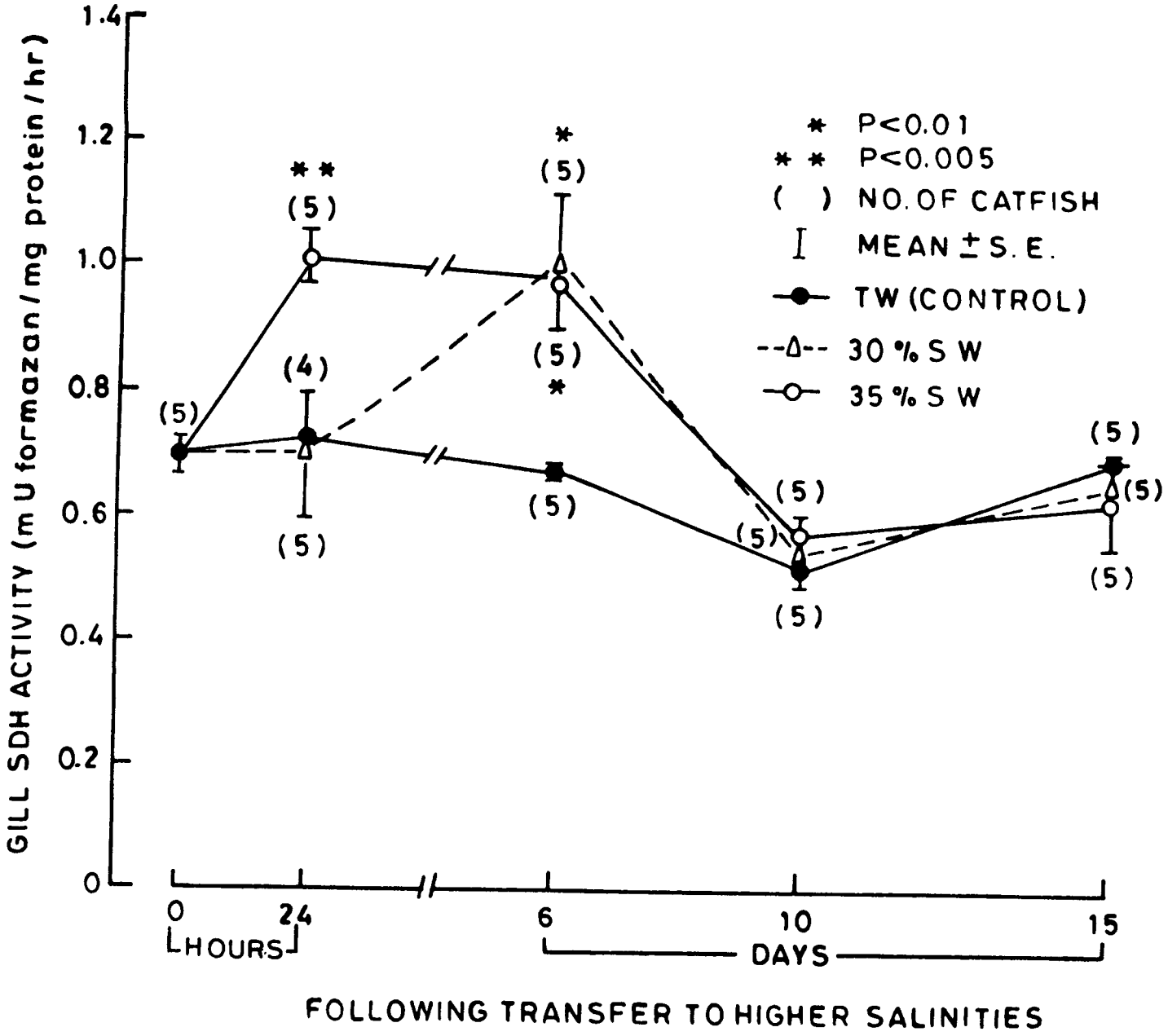
GILL SDH ACTIVITY (m U formazan / mg protein / hr)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
* P<0.01
* * P<0.005
( ) NO. OF CATFISH
MEAN ± S.E.
TW (CONTROL)
30 % S W
35 % S W
(5)
(4)
0
24
6
10
15
HOURS
DAYS
FOLLOWING TRANSFER TO HIGHER SALINITIES

Fig. 6

Fig. 7. Changes in kidney succinic dehydrogenase (SDH) activity of the catfish, *Heteropneustes fossilis* following transfer from tap water (TW) to 30% and 35% sea water (SW).

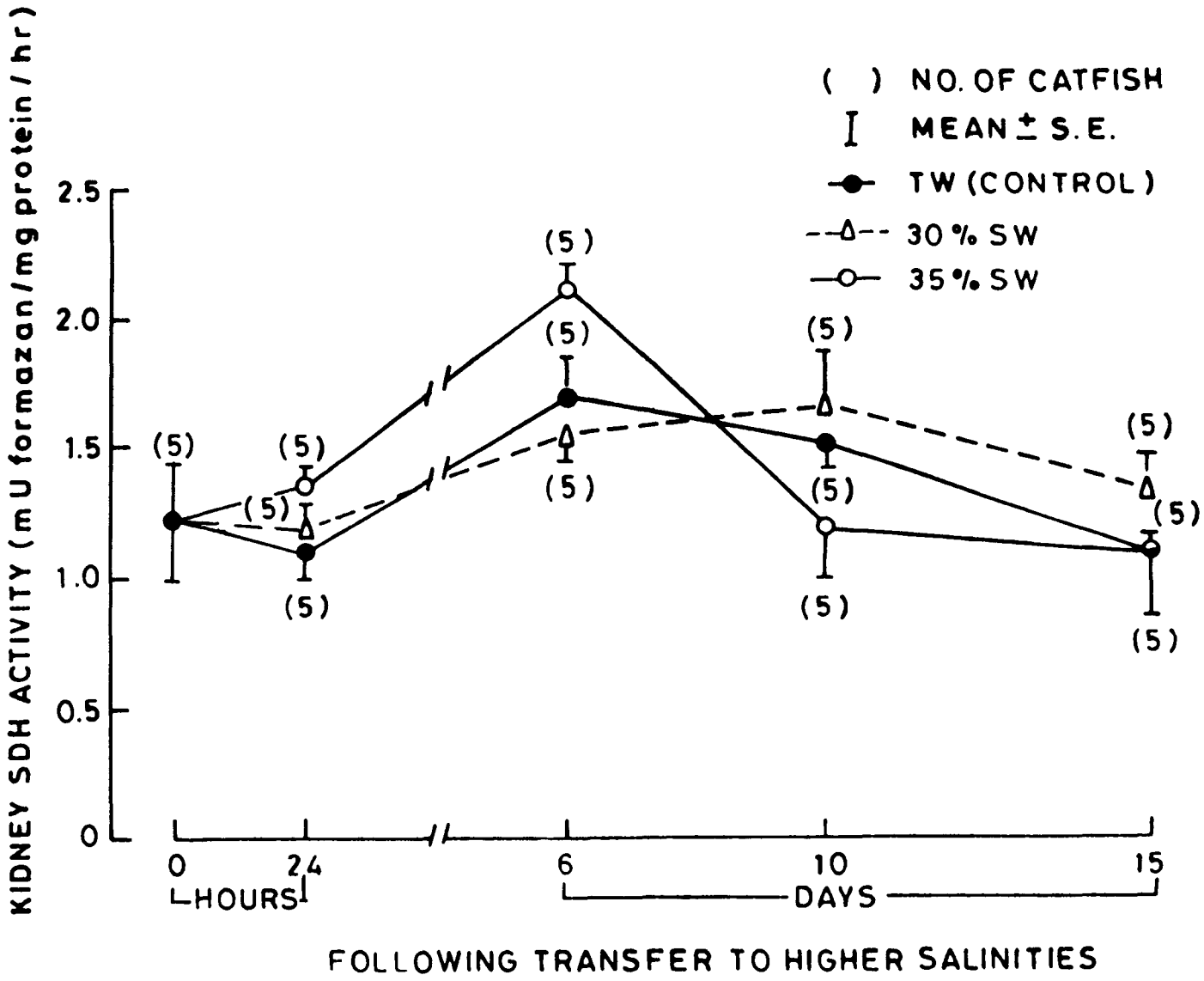
( ) NO. OF CATFISH
MEAN ± S.E.
TW (CONTROL)
30 % SW
35 % SW
KIDNEY SDH ACTIVITY (m U formazan/mg protein / hr)
2.5
2.0
1.5
1.0
0.5
0
(5)
0
24
6
10
15
HOURS
DAYS
FOLLOWING TRANSFER TO HIGHER SALINITIES

Fig.7

CHAPTER IV

ROLE OF CORTISOL IN SEAWATER ADAPTATION AND DEVELOPMENT OF HYPOOSMOREGULATORY MECHANISM IN THE CATFISH, *HETEROPNEUSTES FOSSILIS*

INTRODUCTION

Cortisol (F) is a major hormone facilitating adaptation of teleost in higher salinities. F is also generally regarded to have an effect on carbohydrate metabolism to maintain homeostasis, especially during such metabolic stresses as starvation, spawning, salinity changes and during stress itself (Selye, 1950), thereby ensuring supply of energy and glucose for electrolyte shifts and other transport phenomena (Assem and Hanke, 1979a, b; Hegab and Hanke, 1982, 1984). The increased energetic requirements during seawater (SW) adaptation are often accompanied by the elaboration of sodium/potassium dependent adenosine triphosphatase (Na^+/K^+-ATPase) (Borgatti *et al.*, 1992; Soengas *et al.*, 1995b) and succinic dehydrogenase (SDH) (Sargent *et al.*, 1975; Doneen, 1981; Langdon *et al.* 1984; Madsen *et al.*, 1994) in variety of target organs. These are also known to be mediated by F production (Langdon, *et al.*, 1984; Boeuf and Prunet, 1985;

Madsen and Naamansen, 1989; Olsen *et al.*, 1993; Cornell *et al.*, 1994; Veillette *et al.*, 1995).

Like in other teleosts, F has been found to be involved in osmoionic homeostasis in the catfish, *H. fossilis* based on the following observations. Transfer of the catfish to hyper-osmotic media resulted in activation of corticotrophs in the pituitary (Goswami *et al.*, 1983); daily injection of cortisol acetate (FA) significantly elevated plasma and urine osmolarity and sodium concentration in hypophysectomized catfish maintained in tap water (TW) (Parwez and Goswami, 1985). However, if the fishes were maintained in deionized water, administration of FA had no effect on plasma osmolarity or plasma sodium levels, suggesting that F increases plasma osmotic pressure by stimulating active uptake of salts from the external medium (Parwez and Goswami, 1985). Also, the transfer of the catfish to deionized water results in stimulation of the corticotrophs in the pituitary (Parwez *et al.*, 1994).

Observations in the present investigation (Chapter III) have suggested that the stenohalinity of the catfish may be due to non-reversal of gill functioning from salt-absorption to salt-excretion which possibly results due to low endogenous F levels and hence, it may be suboptimum to bring about an increase in gill Na^+/K^+-ATPase following transfer to

higher salinities (See chapter III). This has also resulted in continuously elevated plasma osmotic pressure due to lack of participation of branchial mechanism. Large number of studies have shown that in teleosts the physiological doses of exogenous F influence branchial Na^+/K^+-ATPase (Hegab and Hanke, 1984; Bisbal and Specker, 1991; McCormick *et al.*, 1991; Cornell *et al.*, 1994; Mancera *et al.* 1994) and SDH activity (Langdon *et al.*, 1984) and improve hypo-ion regulation after SW transfer. Additionally, the depressed ATPase activity and reduced ion efflux rates found in hypophysectomized SW acclimated fish are restored to normal level by F replacement therapy (Pickford *et al.*, 1970; Butler and Carmichael, 1972). Moreover, the effect of exogenous administration of F on glucose metabolism has also been confirmed (Leach and Taylor, 1982; Hegab and Hanke, 1984, 1986; Madsen, 1990a). Therefore, the objective of present investigation is to study the facilitatory role of F during the adaptation process of the catfish, *H. fossilis* in higher salinity following exogenous administration of FA by monitoring changes in plasma osmolarity, plasma glucose, branchial Na^+/K^+-ATPase and SDH activity profile.

MATERIALS AND METHODS

The experiment was conducted on the well-acclimated catfish (mean body weight : 45 g) under laboratory conditions as described in Chapter I. They were fed *ad libitum* daily during acclimation and experimental duration.

FA (Hydrocortisone Acetate, Wyeth Laboratories Limited, India, Batch No. 5C 1011) was diluted with double distilled water (DDW) to the appropriate concentration and injected intramuscularly in the caudal musculature with 1 ml tuberculin syringe fitted with 26 gauge needles at a dose of 10 µg FA/g body weight/100 µl of solvent daily for 5 days. This dose was chosen because of its effectiveness in rectifying deranged osmoregulatory parameters in hypophysectomized catfish (See Parwez and Goswami, 1985).

Experimental Protocol:

Fish were divided into 3 groups. Fish in groups I and II were injected with FA (10 µg/g body weight) daily for 5 days and group III with DDW to serve as control. After 5 days of treatment, the fish of group I were transferred to 30% SW (prepared as described in Chapter II) while groups II and III were transferred to TW. Four to five fish from each group were sampled at 24 hr, 3 and 6 days post-transfer. The blood was obtained from the caudal artery by the method

described earlier. The plasma thus collected was analysed for the estimation of plasma osmolarity and glucose according to the method described in Chapter I. Gills were excised to assay Na^+/K^+-ATPase and SDH according to the method described in Chapter III. The data are presented in the Figs. 1-5.

RESULTS

Plasma osmolarity:

FA treatment for 5 days at a dose of 10 µg/g body wt./day did not affect plasma osmolarity in the group maintained in TW. However, in FA injected fish transferred to 30% SW, a significant increase in plasma osmolarity was observed within 24 hr of transfer ($P<0.005$) which became highly significant on day 3 ($P<0.001$) of the transfer. On the 6th day after transfer, plasma osmolarity levels were more or less the same in all groups (Fig. 1).

Per cent increase in plasma osmolarity with and without FA treatment following transfer to 30% SW (based on the data obtained from Figs. 1 of Chapters II and IV):

The group transferred directly to 30% SW showed 3-4% increase in plasma osmolarity between 3-24 hr which got increased further up to a maximum of 25% on day 6 and then stabilized at 13-14% by day 10-15 when the experiment was

terminated. However, FA treated group showed a much greater increase (18%) in plasma osmolarity at 24 hr which got decreased to 8% on day 6 as compared to 25% observed in directly transferred group (Fig. 2).

Plasma glucose:

FA treated group maintained in TW showed a significant increase in plasma glucose levels within 24 hr ($P<0.05$) following last injection. Except for this, no significant difference in glucose levels was observed in any treatment (Fig. 3).

Gill Na^+/K^+-ATPase:

Fig. 4 shows that the groups treated with FA showed a significant increase in enzyme activity at 24 hr both in TW and 30% SW. The parity with DDW injected control was achieved from 3 days onwards in both groups (Fig. 4).

Gill SDH:

FA treatment significantly decreased SDH activity in both TW and 30% SW maintained groups ($P<0.005$) on day 3 (Fig. 5).

DISCUSSION

There are several studies showing that F influences water and electrolyte exchange in the gills, gut, kidney, urinary bladder and muscle, modifying concomitantly the concentration of electrolytes and osmolarity of blood and urine (Pickford *et al.*, 1970; Hirano and Utida, 1971; Utida *et al.*, 1972; Porthé-Nibelle and Lahlou, 1975; Assem and Hanke, 1981; Langdon *et al.*, 1984; Hegab and Hanke, 1984; Richman and Zaugg, 1987; Madsen, 1990a,b,c). In general, F promotes electrolyte excretion in fish living in hypertonic media and its conservation in hypotonic environment (Maetz, 1969).

The results of the present study do not demonstrate significant change in plasma osmolarity following FA administration in TW maintained catfish. However, a significant increase in plasma osmotic pressure was observed within 24 hr when the catfish pretreated with FA were transferred to 30% SW. This situation in the catfish is similar to that in the yearling coho salmon, *Oncorhynchus kisutch* in FW in which F treatment also did not affect plasma osmolarity, Na^+, K^+, Ca^{2+}, or Mg^{2+} concentration (Redding *et al.*, 1984). The plasma sodium and chloride data of Madsen (1990b) on rainbow trout, *Salmo gairdneri* and the chloride data of Madsen (1990c) on sea trout parr, *Salmo trutta* also

did not indicate changes in the ionic balance of F treated fish in FW. However, Holmes (1959) in *Salmo gairdneri*; Chan *et al.*, (1967) and Hendersan and Chester Jones (1967) in *Anguilla anguilla* and Maetz (1969) in *Fundulus* sp. observed decreased levels of electrolytes in blood and plasma in FW after F treatment. According to these authors, the lowered ion levels in F treated FW fish indicate increased ion-excretion and/or decreased ion-uptake rates. Our data on plasma osmotic pressure of FA-treated fish after transfer to 30% SW corroborate the findings of Folmar and Dickhoff (1980), Hegab and Hanke (1984), Madsen (1990a,b,c) where a significant increase was observed in plasma osmolarity/ion under similar situations.

It is interesting to compare our observations on plasma osmolarity of the two groups of fishes transferred to 30% SW with and without FA pre-treatment (Chapter II, Fig. 1; Chapter IV, Fig, 1). Direct transfer of the catfish to 30% SW without FA-treatment resulted in significant increase of plasma osmolarity from day 3 which remained significantly elevated up to 15 days. Although FA-treatment led an early increase in plasma osmotic pressure *i.e.* 24 hr of post-transfer to 30% SW, in later stages it not only reduced the magnitude of increase (Fig. 2, Chapter IV), but also helped in achieving the parity with control group only within

6 days which could not occur even up to 15 days in the group without FA-treatment. From these observations, it is tempting to suggest that FA-treatment improves the ability of osmoionic regulation after SW transfer (Assem and Hanke, 1981; Hegab and Hanke, 1984; Madsen, 1990a,b,c).

Our data on gill Na^+/K^+-ATPase reveal that FA-treated groups maintain significantly higher enzyme activity both in TW and 30% SW up to 24 hrs following the cessation of the treatment. This further highlights the hypo-osmoregulatory ability of FA and is in accordance with the previous studies on *Cyprinus carpio* (Hegab and Hanke, 1984) ; *Oncorhynchus kisutch* (Richman and Zaugg, 1987); *Salmo gairdneri* (Madsen, 1990a, b); *Salmo trutta* (Madsen, 1990c); *Salmo salar* (Bisbal and Specker, 1991; Cornell *et al.*, 1994). The increased Na^+/K^+-ATPase activity in TW is interesting and supports the idea that increased pre-transfer Na^+/K^+-ATPase level is protective as it reduces the changes in ion-osmotic parameters after transfer to higher salinity (Madsen 1990c). The improved plasma osmotic pressure regulatory ability of the catfish in 30% SW resulting from FA-treatment may be due to changed branchial function from salt-absorption to salt-excretion as is evident from significantly increased Na^+/K^+-ATPase of the fish in 30% SW pretreated with FA (Bisbal and Specker, 1991; Cornell *et al.*, 1994). The treatment of the

fish with FA brings about greater increase of branchial Na^+/K^+-ATPase in SW as compared to TW which is in agreement with earlier studies (Dange, 1986; Madsen, 1990a,b,c). Several studies have shown that F in combination with other hormones was more effective in the development of hypoosmoregulatory ability of fishes (Dange, 1986; Richman and Zaugg, 1987; Madsen, 1990a,b, c). Dange (1986) reported that the combined treatment of F and thyroxine (T_4) was most effective in improving hypoosmoregulatory ability of tilapia, *Oreochromis mossambicus*, while Richman and Zaugg (1987) and Madsen (1990c) reported synergistic effect of F with growth hormone in coho salmon, *Oncorhynchus kisutch* and sea trout parr, *Salmo trutta trutta* respectively. It would be meaningful to see the combined effect of FA with T_3 and/or T_4 on the hypoosmoregulatory ability of *H. fossilis* in future studies.

The present study demonstrates no significant change in SDH activity in FA-treated groups in the initial phase of 24 hr. However, it showed a significant decline only on day 3. The exact role played by SDH during the adaptation process of the catfish in different salinities cannot be predicted at the moment. Langdon *et al.* (1984) did not observe any significant change in SDH activity following F treatment in the gill homogenate of 10-month-old parr and 12-month-old

presmolts of Atlantic salmon, *Salmo salar*.

Most of the reports on the effect of F administration in fish deal with its action on carbohydrate metabolism (Boon *et al.*, 1991). The present investigation reveals a significant increase in plasma glucose levels in FA treated fish in TW while the levels remained more or less constant when they were transferred to 30% SW. The hyperglycemic effect of FA treatment as observed in TW corroborates the earlier findings on roach, *Leuciscus rutilus* (Müller and Hanke, 1974), air-breathing teleost, *Channa punctatus* (Gill and Khanna, 1975), eel, *Anguilla japonica* (Chan and Woo, 1978), Killifish, *Fundulus heteroclitus* (Leach and Taylor, 1982), rainbow trout, *Salmo gairdneri* (Barton *et al.*, 1987; Madsen, 1990a). FA-induced hyperglycemia in catfish may result from inhibitory effects of hormone on glucose oxidation and utilization in peripheral tissues (Boon *et al.*, 1991). Another explanation is that FA may provide glucose either through glycogenolysis or through gluconeogenesis, since it has catabolic (Barton *et al.*, 1987) as well as anabolic effects (Chan and Woo, 1978; De La Higuera and Cardenas, 1986). The unchanged plasma glucose levels in FA treated fish in 30% SW (Fig. 3) may reflect increased tissue utilization of glucose. Suggestion of similar kind has been made by Vijayan *et al.*, (1991) when they found either

unchanged or reduced plasma glucose in F treated brook trout, *Salvelinus fontinalis*. While observations on plasma glucose of FA treated fish transferred to 30% SW are in line with those of Hegab and Hanke (1984) for carp, *Cyprinus carpio*, they differ with those of Hegab and Hanke (1984) on tilapia, *Sarotherodon mossambicus* and Madsen (1990a) on rainbow trout, *Salmo gairdneri* where plasma glucose registered significant decrease.

From the aforesaid description, it may be concluded that FA treatment prepares TW adapted catfish to acclimate to hyper-osmotic environment. The experimental treatment may mobilise both the putative ion-excreting machinery and the energy for oxidative metabolism in transporting epithelia in the catfish (see also Madsen 1990a). A possible synergistic action of F with other hormones such as T_3 and T_4 to improve the hypoosmotic ability of the catfish needs to be investigated.

Fig. 1. Changes in plasma osmolarity of the catfish, *Heteropneustes fossilis* after cortisol acetate (FA) treatment in tap water (TW) and 30% sea water (SW). Asterisks denote significant difference in values compared to control group.

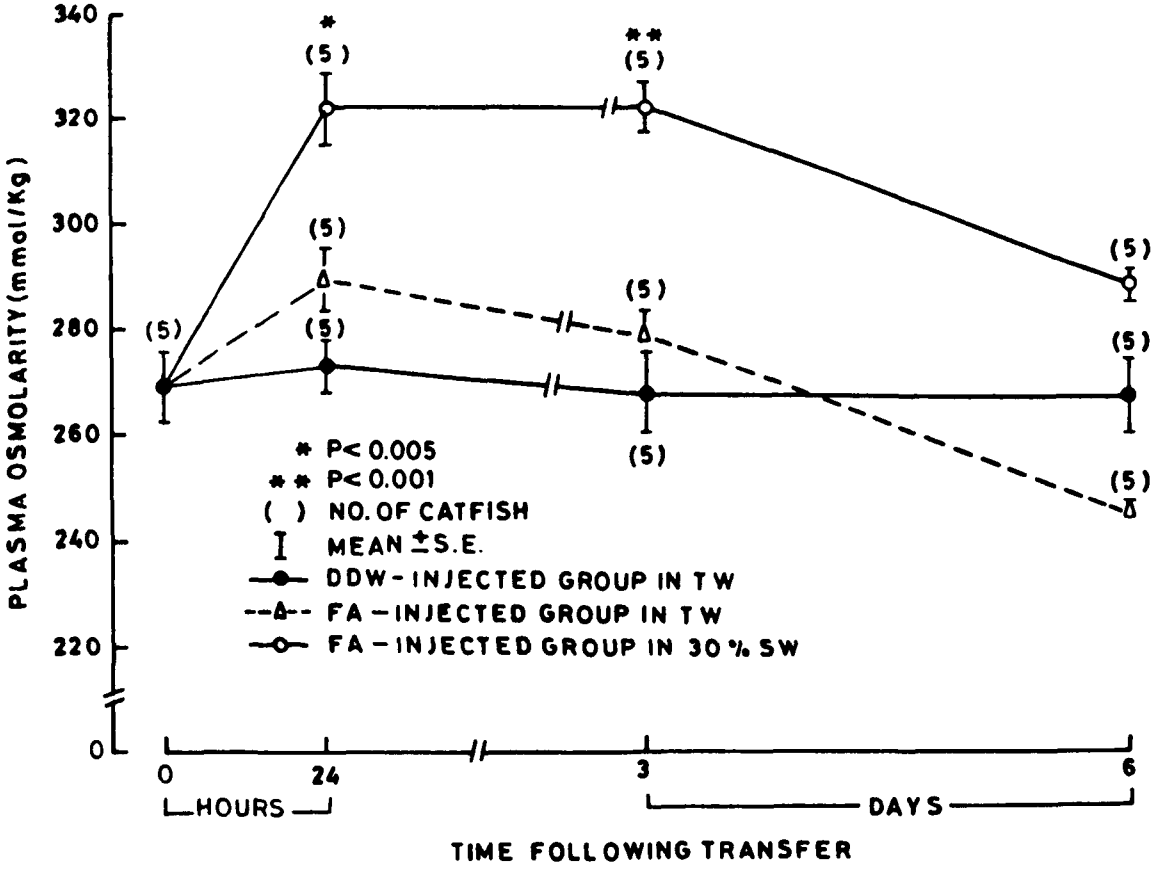
PLASMA OSMOLARITY (mmol/Kg)
340
320
300
280
260
240
220
0
*
**
(5)
* P< 0.005
** P< 0.001
( ) NO. OF CATFISH
MEAN ± S.E.
DDW - INJECTED GROUP IN TW
FA - INJECTED GROUP IN TW
FA - INJECTED GROUP IN 30 % SW
0
24
3
6
HOURS
DAYS
TIME FOLLOWING TRANSFER

Fig.1

Fig. 2. Per cent increase in plasma osmolarity of the catfish, *Heteropneustes fossilis* with and without cortisol acetate (FA) treatment in 30% sea water (SW).

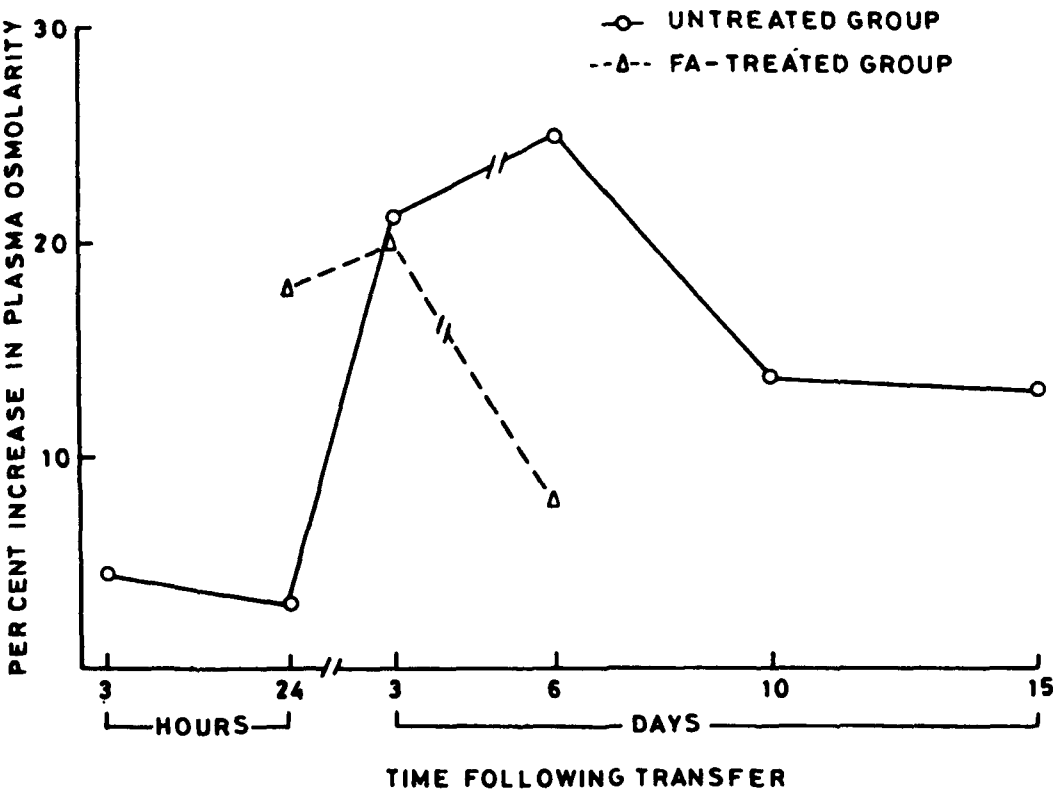
UNTREATED GROUP
FA-TREATED GROUP
PER CENT INCREASE IN PLASMA OSMOLARITY
30
20
10
3
24
3
6
10
15
HOURS
DAYS
TIME FOLLOWING TRANSFER

Fig. 2

Fig. 3. Changes in plasma glucose concentration of the catfish, *Heteropneustes fossilis* after cortisol acetate (FA) treatment in tap water (TW) and 30% sea water (SW). Asterisk denotes significant difference in values compared to control group.

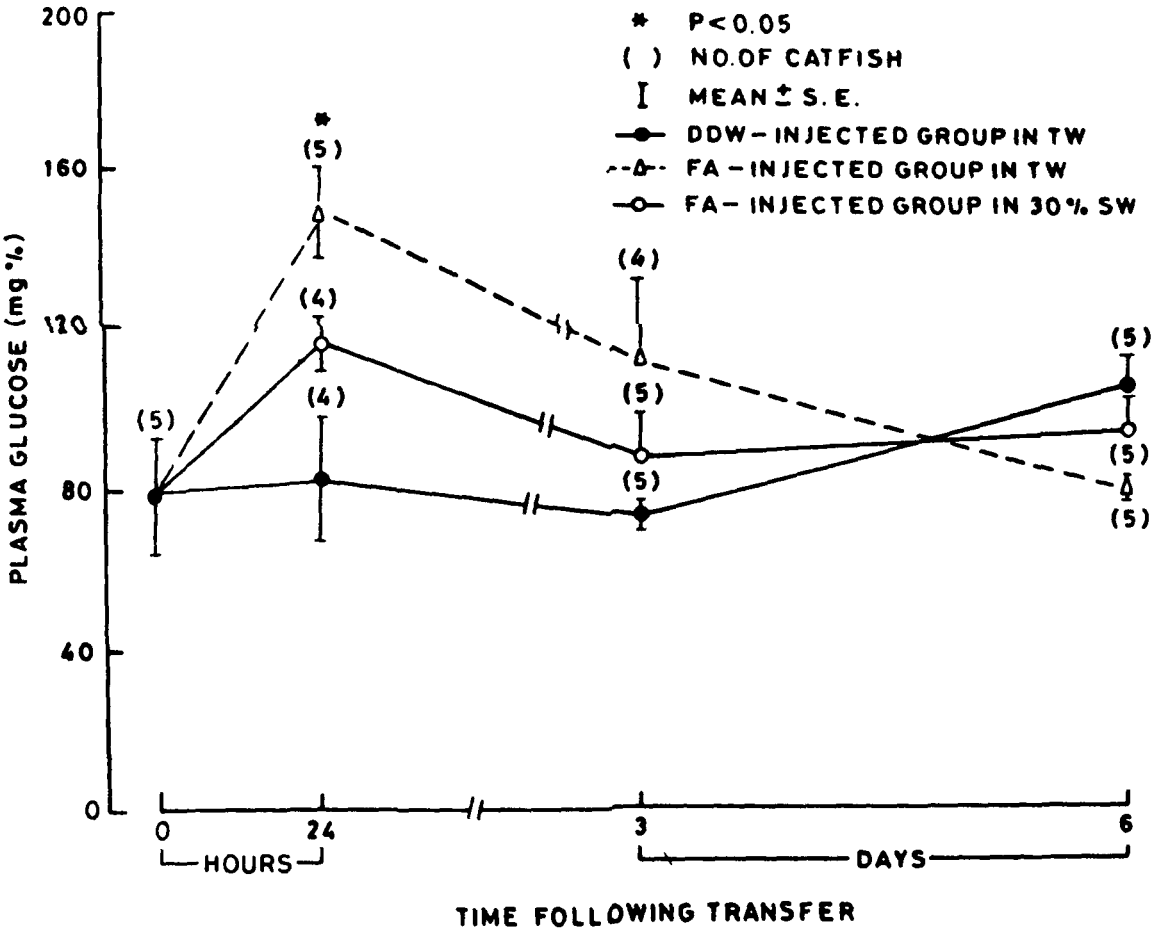
* P<0.05
( ) NO.OF CATFISH
I MEAN ± S.E.
DDW-INJECTED GROUP IN TW
FA-INJECTED GROUP IN TW
FA-INJECTED GROUP IN 30% SW
PLASMA GLUCOSE (mg %)
200
160
120
80
40
0
(5)
(4)
(4)
(5)
(4)
(5)
(5)
(5)
(5)
0
24
3
6
HOURS
DAYS
TIME FOLLOWING TRANSFER

Fig. 3

Fig. 4. Changes in gill sodium/potassium dependent adenosine triphosphatase (Na^+/K^+-ATPase) activity of the catfish, *Heteropneustes fossilis* after cortisol acetate (FA) treatment in tap water (TW) and 30% sea water (SW). Asterisks denote significant difference in values compared to control group.

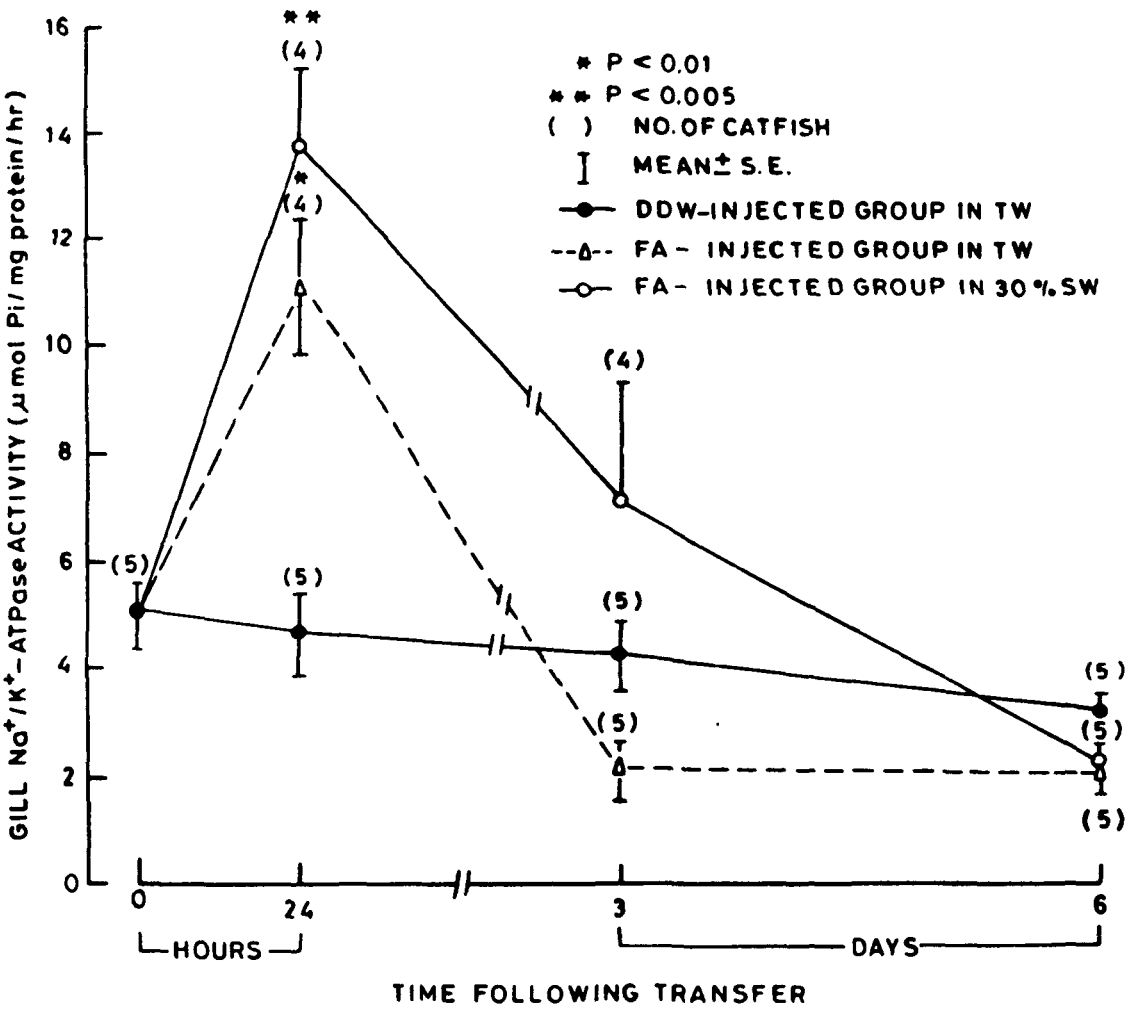
GILL Na+/K+-ATPase ACTIVITY (μ mol Pi/ mg protein/ hr)
16
14
12
10
8
6
4
2
0
** P < 0.01
** P < 0.005
( ) NO. OF CATFISH
MEAN ± S.E.
DDW-INJECTED GROUP IN TW
FA - INJECTED GROUP IN TW
FA - INJECTED GROUP IN 30 % SW
(4)
(4)
(4)
(5)
(5)
(5)
(5)
(5)
(5)
0
24
3
6
HOURS
DAYS
TIME FOLLOWING TRANSFER

Fig 4

Fig. 5. Changes in gill succinic dehydrogenase (SDH) activity of the catfish, *Heteropneustes fossilis* after cortisol acetate (FA) treatment in tap water (TW) and 30% sea water (SW). Asterisks denote significant difference in values compared to control group.

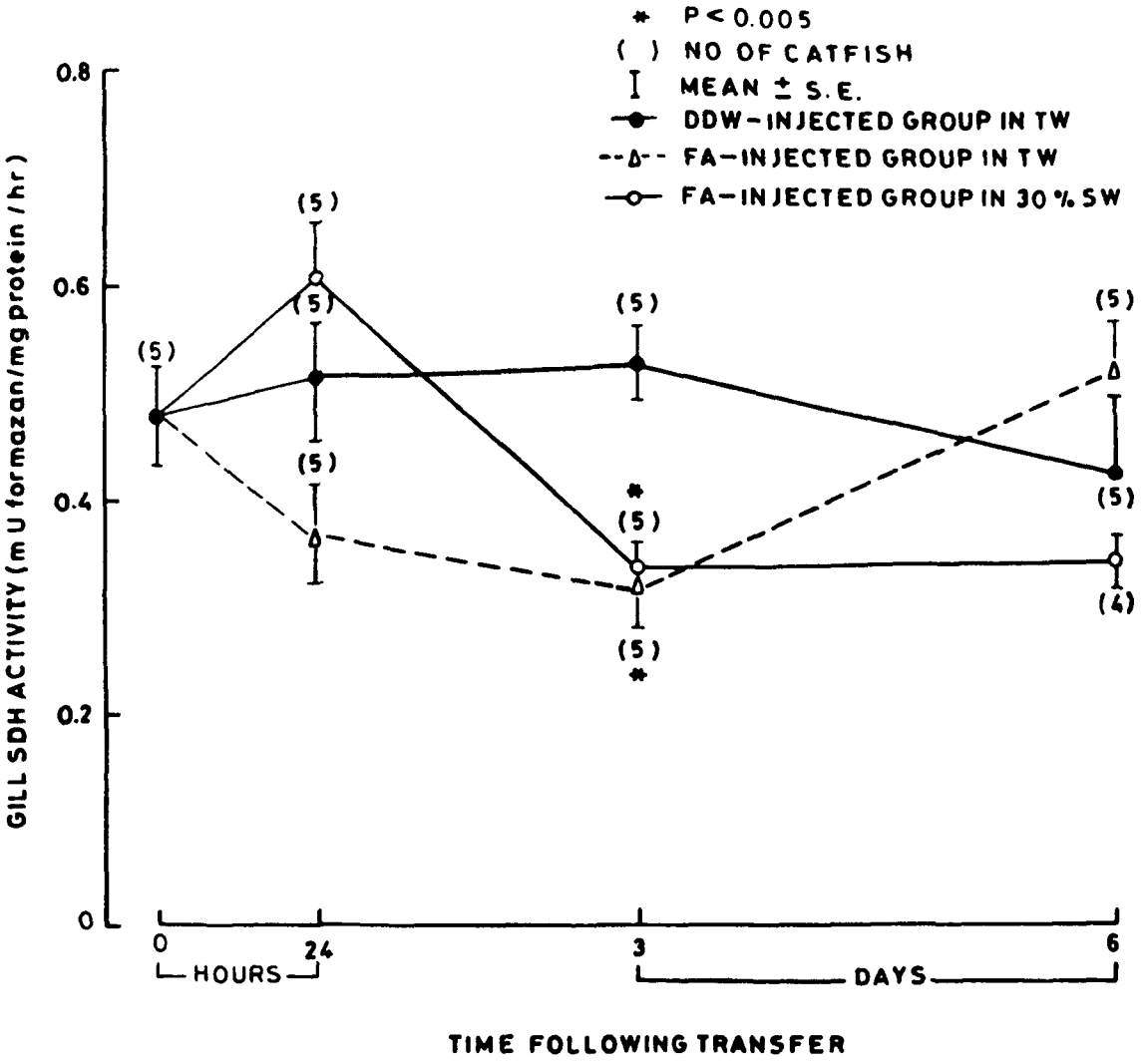
* P < 0.005
( ) NO OF CATFISH
MEAN ± S.E.
DDW-INJECTED GROUP IN TW
FA-INJECTED GROUP IN TW
FA-INJECTED GROUP IN 30 % SW
GILL SDH ACTIVITY (m U formazan/mg protein / hr)
0.8
0.6
0.4
0.2
0
(5)
(4)
*
0
24
3
6
HOURS
DAYS
TIME FOLLOWING TRANSFER

Fig.5

REFERENCES

Alliot, E. (1982). Aspects nutritionnels chez le loup (*Dicentrarchus labrax*) en milieu contrôlé. Influences des régimes alimentaires et des facteurs de l'environment sur le bilan azoté. Thése d' élat Université AIX-MAR. SEILLE II.

Alliot, E., Djabal, M., Pastoureaud, A. and Thebault, H. (1984). Changes in the biochemical composition of tissues in juvenile sea bass during forced starvation. Biochem. Syst. Ecol. 12: 209-213.

Assem, H. and Hanke, W. (1979a). Concentration of carbohydrates during osmotic adjustment of the euryhaline teleost, *Tilapia mossambica*. Comp. Biochem. Physiol. 64A: 5-16.

Assem, H. and Hanke, W. (1979b). Volume regulation of muscle cells in the euryhaline teleost, *Tilapia mossambica*. Comp. Biochem. Physiol. 64A: 17-23.

Assem, H. and Hanke, W. (1981). Cortisol and osmotic adjustment of the euryhaline teleost, *Sarotherodon mossambicus*. Gen. Comp. Endocrinol. 43: 370-380.

Bailey, J.L. (1962). Estimation of protein. *In*" Techniques in Protein Chemistry." Chapter 11: Miscellaneous Analytical Methods, pp. 293-294.

Barton, B.A. and Iwama, G.K. (1991). Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. Ann. Rev. Fish Dis. 1: 3-26.

Barton, B.A., Peter, R.E. and Paulencu, C.R. (1980). Plasma cortisol levels of fingerling rainbow trout (*Salmo gairdneri*) at rest, and subjected to handling, confinement, transport, and stocking. Can. J. Fish. Aquat. Sci. 37: 805-811.

Barton, B.A., Schreck, C.B. and Barton, L. D. (1987). Effects of chronic cortisol administration and daily acute stress on growth, physiological conditions and stress responses in juvenile rainbow trout. Dis. Aquat. Org. 2: 17-186.

Barton, B.A., Schreck, C.B. Ewing, R.D., Hemmingsen, A.R. and Patino, R. (1985). Changes in plasma cortisol during stress and smoltification in coho salmon, *Oncorhynchus kisutch*. Gen. Comp. Endocrinol. 59: 468-471.

Barton, B.A., Schreck, C.B. and Sigismondi, L.A. (1986). Multiple acute disturbances evoke cumulative physiological response in juvenile chinook salmon. Trans. Am. Fish. Soc. 115: 245-251.

Bashamohideen, M. and Parvatheswararao, V. (1972). Adaptation to osmotic stress in the freshwater euryhaline teleost *Tilapia mossambica*. IV. Changes in blood glucose, liver glycogen and muscle glycogen levels. Mar. Biol. 16: 68-74.

Biron, M. and Benfey, T.J. (1994). Cortisol, glucose and hematocrit changes during acute stress, cohort sampling, and the diel cycle in diploid and triploid brook trout (*Salvelinus fontinalis* Mitchill). Fish Physiol. Biochem. 13: 153-160.

Bisbal, G.A. and Specker, J.L. (1991). Cortisol stimulates hypoosmoregulatory ability in Atlantic salmon, *Salmo salar*. L. J. Fish Biol. 39: 421-432.

Black, D. and Love, M.R. (1986). The sequential mobilization and restoration of energy reserves in the tissues of Atlantic cod during starvation and refeeding. J. Comp. Physiol. 156B: 469-479.

Boeuf, G. and Lasserre, P. (1979). Aspect de la regulation osmotique chez le bar juvenile (*Dicentrarchus labrax*) en elevage et introduit dans les lagunes amenagrees de certex. Bassin d'Arcachon (Gironde). Actes de Colloque CNEXO (France) 7: 673-688.

Boeuf, G. and Le Bail, P.Y. (1990). Growth hormone and thyroid hormones levels during smolting in different populations of Atlantic salmon. *In* "Progress in Comparative Endocrinology". 342: 193-197.

Boeuf, G., Le Bail, P.Y. and Prunet, P. (1989). Growth hormone and thyroid hormones during Atlantic salmon, *Salmo salar* L., smolting and after transfer to sea water. Aquaculture 82: 257-268.

Boeuf, G. and Prunet, P. (1985). Measurements of gill (Na^+-K^+)-ATPase activity and plasma thyroid hormones during smoltification in Atlantic salmon (*Salmo salar* L.). Aquaculture 45: 111-119.

Booke, H.E., Hollender, B. and Lutterbie, G. (1978). Sodium bicarbonate, an inexpensive fish anaesthetic for field use. Prog. Fish-Cult. 40: 11-13.

Boon, J., Thillart, G.E.E.J.M. and Addink, A.D. F. (1991). The effects of cortisol administration on intermediary metabolism in teleost fish. Comp. Biochem. Physiol. 100A: 47-53.

Borgatti, A. R., Pagliarini, A. and Ventrella, V. (1992). (Gill Na^+/K^+)-ATPase involvement and regulation during salmonid adaptation to salt water. Comp. Biochem. Physiol. 102A: 637-643.

Butler, D.G. and Carmichael, F.J. (1972). Na^+-K^+-ATPase activity in eel (*Anguilla rostrata*) gills in relation to changes in environmental salinity: role of adrenocortical steroids. Gen. Comp. Endocrinol. 19: 421-427.

Chan, D.K.O., Chester, I., Henderson, I.W. and Rankin, J.C. (1967). Studies on the experimental alteration of water and electrolyte composition of the eel (*Anguilla anguilla* L.). J. Endocrinol. 37: 297-317.

Chan, D.K.O. and Woo, N.Y.S. (1978). Effect of cortisol on the metabolism of the eel, *Anguilla japonica*. Gen. Comp. Endocrinol. 35: 205-215.

Chavin, W. and Young, J.E. (1970) . Factors in determination of normal serum glucose levels of goldfish, *Carassius auratus* L. Comp. Biochem. Physiol. 33: 629-653.

Collins, A.L. and Anderson, T.A. (1995). The regulation of endogenous energy stores during starvation and refeeding in the somatic tissues of the golden perch. J. Fish Biol. 47: 1004-1015.

Conte, F.P. (1969). The biochemical aspects of salt secretion. *In* "Fish in Research" (O.W. Neuhaus and J.E. Halver, eds.), pp. 105-120. Academic Press, New York.

Cornell, S.C., Portesi, D.M., Veillette, P.A., Sundell, K. and Specker, J.L. (1994). Cortisol stimulates intestinal fluid uptake in Atlantic salmon (*Salmo salar*), in the post-smolt stage. Fish Physiol. Biochem. 13: 183-190.

Cowey, C.B. and Sargent, J.W. (1979). Nutrition. *In* " Fish Physiology" (W.S. Hoar and D.J. Randall, eds.), Vol. 8, pp. 1-69. Academic Press, New York and London.

Dange, A.D. (1986). Branchial Na^+/K^+-ATPase activity in freshwater or saltwater acclimated tilapia, *Oreochromis* (*Sarotherodon*) *mossambicus*: Effect of cortisol and thyroxine. Gen. Comp. Endocrinol. 62: 341-343.

Das, P and Kapoor, D. (1996). Fish biodiversity conservation in India. The Fourth Indian Fisheries Forum (P.U. Verghese ed.), pp. 42-50. Nov. 24-28, 1996, Manglore, India.

Dave, G., Johansson-Sjobeck, M.L., Larsson, A., Lewander, K. and Lidman, U. (1975). Metabolic and hematological effects of starvation in the European eel, *Anguilla Anguilla* L.-I. Carbohydrate, lipid, protein and inorganic ion metabolism. Comp. Biochem. Physiol. 52A: 423-430.

Davis, K.B., Parker, N.C. and Suttle, M.A. (1982). Plasma corticosteroids and chlorides in striped bass exposed to tricaine methanesulfonate, quinaldine, metomidate, and salt. Prog. Fish-Cult. 44: 205-207.

Davis, K.B., Torrance, P., Parker, N.C. and Suttle, M.A. (1985). Growth, body composition and hepatic tyrosine aminotransferase activity in cortisol-fed channel catfish, *Ictalurus punctatus*. J. Fish Biol. 27: 177-184.

De La Higuera, M. and Cardenas, P. (1986). Hormonal effects on gluconeogenesis from (U-^{14}C) glutamate in rainbow trout (*Salmo gairdneri*). Comp. Biochem. Physiol. 85B: 517-521.

De Renzis, G. and Bornancin, M. (1984). Ion transport and gill ATPases. *In* " Fish Physiology" (W.S. Hoar and D.J. Randall, eds.), Vol. XB, pp. 65-104 Academic Press, New York and London.

Dickhoff, W.W. (1992). Seawater adaptation. *In* "Recent Advances in Aquaculture IV" (J.F. Muir and R.J. Roberts, eds.), pp. 152-192. Blackwell Scientific Publication, Oxford.

Dickhoff, W.W., Folmar, L.C., Mighell, J.L. and Mahnken, C.V.W. (1982). Plasma thyroid hormones during smoltification of yearling and underyearling coho salmon and yearling chinook salmon and steelhead trout. Aquaculture 28: 39-48.

Donaldson, E.M. (1981). The pituitary-interrenal axis as an indicator of stress in fish. *In* "Stress and Fish" (A.D. Pickering, ed.), pp. 11-47. Academic Press, London.

Doneen, B.A. (1981). Effects of adaptation to sea water, 170% sea water and to fresh water on activities and subcellular distribution of branchial Na^+-K^+-ATPase, low and high affinity Ca^{++}-ATPase and ouabain-insensitive ATPase in *Gillichthys mirabilis*. J. Comp. Physiol. 145: 51-61.

Einarsdottir, I.E. and Nilssen, K.J. (1996). Stress responses of Atlantic salmon (*Salmo salar* L.) elicited by water level reduction in rearing tanks. Fish Physiol. Biochem. 15:363-370.

Epstein, F.H., Katz, A.I. and Pickford, G.E. (1967). Sodium and potassium activated adenosine triphosphatase of gills : role in adaptation of teleost to salt water. Science 156: 1245-1247.

Epstein, F.H., Manitius, A., Weinstein, E., Katz, A.I. and Pickford, G.E. (1969). Sodium and potassium-activated adenosine triphosphatase in kidneys of *Fundulus heteroclitus* adapted to fresh and salt water. Yale J. Biol. Med. 41: 388-393.

Evans, D.H. (1980). Osmotic and ionic regulation by freshwater and marine fishes. *In* "Environmental Physiology of Fishes" (M.A. Ali, ed.), pp. 93-121. Plenum Press, New York.

Evans, D.H. (1984). The roles of gill permeability and transport mechanisms in euryhalinity. *In* "Fish Physiology" (W.S. Hoar and D.J. Randall, eds.), Vol. XB, pp. 239-283. Academic Press New York and London.

Evans, D.H., Mallery, C.H. and Kravitz, L. (1973). Sodium extrusion by a fish acclimated to sea water : physiological and biochemical description of a Na for K exchange system J. Exp. Biol. 58: 627-636.

Farghaly, A.M., Ezzat, A.A. and Shabana, M.B. (1973). Effect of temperature and salinity changes on the blood characteristics of *Tilapia zilli* in Egyptian littoral lakes. Comp. Biochem. Physiol. 46A: 183-193.

Febry, R. and Lutz, P. (1987). Energy partitioning in fish: the activity-related cost of osmoregulation in a euryhaline cichlid. J. Exp. Biol. 128: 63-85.

Finstad, B., Nilssen, K.J. and Arnesen, A.M. (1989). Seasonal changes in the seawater tolerance of Arctic charr (*Salvelinus alpinus*). J. Comp. Physiol. 159B: 371-378.

Finstad, B., Staurnes, M. and Reite, O.B. (1988). Effect of low temperature on seawater tolerance in rainbow trout, *Salmo gairdneri*. Aquaculture 72: 319-328.

Finstad, B, and Thomassen, M.S. (1991). Does dietary lipid composition affect the osmoregulatory ability of rainbow trout (*Oncorhynchus mykiss*) at high and low temperature? Comp. Biochem. Physiol. 99A: 463-471.

Fletcher, G.J. (1984). Plasma glucose and plasma fatty acid levels of *Limanda limanda* (L.) in relation to season, stress, glucose load and nutritional state. J. Fish Biol. 25: 629-648.

Folmar, L.C. and Dickhoff, W.W. (1980). The parr-smolt transformation (smoltification) and seawater adaptation in salmonids. A review of selected literature. Aquaculture 21:1-37.

Folmar, L.C. and Dickhoff, W.W. (1981). Evaluation of some physiological parameters as predictive indices of smoltification. Aquaculture 23: 309-324.

Foskett, J.K., Bern, H.A., Machen, T.E. and Conner, M. (1983). Chloride cells and the hormonal control of teleost fish osmoregulation. J. Exp. Biol. 106: 255-281.

Foster, A.R., Houlihan, D.F. and Hall, S.J. (1993). Effects of nutritional regime on correlates of growth rate in juvenile atlantic cod (*Gadus morhua*): Comparison of morphological and biochemical measurements. Can. J. Fish. Aquat. Sci. 50: 502-512.

Fryer, J.N. (1975). Stress and adrenocorticosteroid dynamics in the goldfish, *Carassius auratus*. Can. J. Zool. 5: 1012-1020.

Furspan, P., Prange, H.D. and Munck, O. (1984). Energetics of osmoregulation in the catfish, *Ictalurus nebulosus* and *I. punctatus*. Comp. Biochem. Physiol. 77A: 773-778.

Gaumet, F., Boeuf, G. Severe, A., Le Roux, A. and Mayer-Gostan, N. (1995). Effects of salinity on the ionic balance and growth of juvenile turbot. J. Fish Biol. 47: 865-876.

Gill, T.S. and Khanna, S.S. (1975). Effects of cortisol administration upon a freshwater teleost, *Channa punctatus*. Zool. Beitr. 25: 191-198.

Goswami, S.V., Parwez, I. and Sundararaj, B.I. (1983). Some aspects of osmoregulation in a stenohaline freshwater catfish, *Heteropneustes fossilis* (Bloch), in different salinities. J. Fish. Biol. 23: 475-487.

Grau, E.G., Nishioka, R.S. and Bern, H.A. (1980). Effects of seawater entry on thyroid function in the coho salmon, *Oncorhynchus kisutch*. Amer. Zool. 20: 857.

Hanke, W. (1991). Mechanism of osmotic adaptation in fresh water teleost. Fisch. Forsch. 29: 15-19.

Hegab, S.A. and Hanke, W. (1982). Electrolyte changes and volume regulatory processes in the carp (*Cyprinus carpio*) during osmotic stress. Comp. Biochem. Physiol. 71A: 157-164.

Hegab, S.A. and Hanke, W. (1984). The significance of cortisol for osmoregulation in carp (*Cyprinus carpio*) and tilapia (*Sarotherodon mossambicus*). Gen. Comp. Endocrinol. 54: 409-417.

Hegab, S.A. and Hanke, W. (1986). Electrolyte changes, cell volume regulation and hormonal influences during acclimation of rainbow trout (*Salmo gairdneri*) to salt water. Comp. Biochem. Physiol. 83A: 47-52.

Hendersan, I.W. and Chester Jones, I. (1967). Endocrine influences on the net extrarenal fluxes of sodium and potassium in the European eel (*Anguilla anguilla* L.). J. Endocrinol. 37: 319-325.

Hirano, T., Morisawa, M., Ando, M. and Utida, S. (1976). Adaptive changes in ion and water transport mechanism in the eel intestine. *In* "Intestinal Ion Transport" (J.W.L. Robinson, ed.), pp. 301-317. Medical and Technical Pub. Co., Lancaster.

Hirano, T. and Utida, S. (1971). Plasma cortisol concentration and rate of intestinal water absorption in the eel, *Anguilla japonica*. Endocrinol. Japon. 18: 47-52.

Holmes, W.N. (1959). Studies on the hormonal control of sodium metabolism in the rainbow trout (*Salmo gairdneri*). Acta Endocrinol. 31: 587-602.

Holmes, W.N. and Donaldson, E.M. (1969). The body compartments and the distribution of electrolytes. *In* "Fish Physiology" (W.S. Hoar and D.J. Randall, eds.), Vol. I, pp. 1-89. Academic Press, New York and London.

Holmes, W.N. and Lockwood, C.N. (1970). Some aspects of osmoregulation in the barred surfperch (*Amphistichus argenteus*). J. Zool. Lond. 161: 1-14.

Houston, A.H., Madden, J.A., Woods, R.J. and Miles, H.M. (1971). Some physiological effects of handling and tricain methanesulphonate anesthetization upon the brook trout, *Salvelinus fontinalis*. J. Fish. Res. Bd. Can. 28: 625-633.

Houston, A.H. and Mearow, K.M. (1982). Branchial and renal Na^+, K^+-ATPase and carbonic anhydrase activities in the eurythermal freshwater teleost, *Carassius auratus* L. Comp. Biochem. Physiol. 71A: 175-180.

Hutchinson, S. and Hawkins, L.E. (1990). The influence of salinity on water balance in O-group flounders, *Platichthys flesus* (L.). J. Fish Biol. 36: 751-764.

Hwang, P.P., Sun, C.M. and Wu, S.M. (1989). Changes of plasma osmolality, chloride concentration and gill Na-K-ATPase activity in tilapia *Oreochromis mossambicus* during seawater acclimation. Mar. Biol. 100: 295-299.

Hyvarinen, A. and Nikkila, E.A. (1962). Method using O-Toluidine. Clin. Chim. Acta 7:140.

Ince, B.W. and Thorpe, A. (1975). Hormonal and metabolic effects on plasma free fatty acids in the northern pike, *Esox lucius* L. Gen. Comp. Endocrinol. 27: 144-152.

Ishiota, H. (1980). Stress reactions induced by environmental salinity changes in red sea bream. Bull. Jap. Soc. Sci. Fish. 46: 1323-1331.

Jackson, A.J. (1981). Osmotic regulation in rainbow trout (*Salmo gairdneri*) following transfer to seawater. Aquaculture. 24: 143-151.

Jacob, W.F. and Taylor, M.H. (1983). The time course of seawater acclimation in *Fundulus heteroclitus* L. J. Exp. Zool. 228: 33-39.

Jampol, L.M. and Epstein, F.H. (1970). Sodium-potassium-activated adenosine triphosphatase and osmotic regulation by fishes. Amer. J. Physiol. 218: 607-611.

Johnson, D.W. (1973). Endocrine control of hydromineral balance in teleosts. Amer. Zool. 13: 799-818.

Jürss, K., Bittorf, Th. and Völker, Th. (1982a). EinfluB der Ernahrung auf die Na/K-ATPase-Aktivitat in den kiemen der Regenbog enforelle (*Salmo gairdneri* Richardson). Biol. Rdsch. 20: 223-225.

Jürss, K., Bittorf, Th. and Völker, Th. (1984a). Biochemical investigations on salinity and temperature acclimation of the rainbow trout, *Salmo gairdneri* (Richardson). Zool. Jahrb. Physiol. 88: 67-81.

Jürss, K., Bittorf, Th. and Völker, Th. (1985). Influence of salinity and ratio of lipid to protein in diets on certain enzyme activities in rainbow trout (*Salmo gairdneri* Richardson). Comp. Biochem. Physiol. 81B: 73-79.

Jürss, K., Bittorf, Th. and Völker, Th. (1986). Influence of salinity and food deprivation on growth, RNA/DNA ration and certain enzyme activities in rainbow trout (*Salmo gairdneri* Richardson). Comp. Biochem. Physiol. 83B: 425-433.

Jürss, K., Bittorf, Th., Völker, Th. and Wacke, R. (1982b). Experimental studies on biochemical and physiological differences between the three morphs of the three spined stickleback, *Gasterosteus aculeatus* L. I. gill Na^+/K^+-ATPase, muscle alanine aminotransferase and muscle aspartate aminotransferase activities. Zool. Jb. Physiol. 86: 267-272.

Jürss, K., Bittorf, Th., Völker, Th. and Wacke, R. (1983). Influence of nutrition on biochemical sea water adaptation of the rainbow trout (*Salmo gairdneri* Richardson). Comp. Biochem. Physiol. 75B: 713-717.

Jurss, K., Bittorf, Th., Volker, Th. and Wacke, R. (1984b). Biochemical investigation into the influence of environmental salinity on starvation of tilapia (*Oreochromis mossambicus*). Aquaculture 40: 171-182.

Jürss, K., Bittorf, Th., Völker, Th. and Wacke, R. (1987). Effects of temperature, food deprivation and salinity on growth, RNA/DNA-ratio and certain enzyme activities in rainbow trout (*Salmo gairdneri* Richardson). Comp. Biochem. Physiol. 87B: 241-253.

Kamiya, M. (1972a). Sodium-potassium-activated adenosine-triphosphatase in isolated chloride cells from eel gills. Comp. Biochem. Physiol. 43B: 611-617.

Kamiya, M. (1972b). Hormonal effects on Na-K-ATPase activity in the gill of Japanese eel, *Anguilla japonica*, with special reference to seawater adaptation. Endocrinol. Japon. 19: 489-493.

Kamiya, M. and Utida, S. (1968). Changes in activity of sodium-potassium-activated adenosine-triphospha- ase in gills during adaptation of the Japanese eel to sea water. Comp. Biochem. Physiol. 26: 675-685.

Keys, A.B. and Willmer, E.N. (1932). "Chloride-secreting cells" in the gills of fishes with special reference to the common eel. J. Physiol. (Lond.) 76: 368-378.

Kirschner, L.B. (1993). The energetics of osmotic regulation in ureotelic and hypoosmotic fishes. J. Exp. Zool. 267: 19-26.

Knoph, M.B. (1995). Effects of metomidate anaesthesia or transfer to pur sea water on plasma parameters in ammonia-exposed Atlantic salmon (*Salmo salar* L.) in sea water. Fish Physiol. Biochem. 14: 103-109.

Knoph, M.B. and Olsen, Y.A. (1994). Subacute toxicity of ammonia to Atlantic salmon (*Salmo salar* L.) in seawater:effects on water and salt balance, plasma cortisol and plasma ammonia levels. Aquat. Toxicol. 30: 295-310.

Korcock, D.E., Houston, A.H. and Gray, J.D. (1988). Effects of sampling conditions on selected blood variables of rainbow trout, *Salmo gairdneri* Richardson. J. Fish. Biol. 33: 319-330.

Krogh, A. (1939). "Osmotic regulation in Aquatic Animals". Cambridge Univ. Press.

Kültz, D., Bastrop, R., Jürss, K. and Siebers, D. (1992). Mitochondria-Rich (MR) cells and the activities of the Na^+/K^+-ATPase and carbonic anhydrase in the gill and opercular epithelium of *Oreochromis mossambicus* adapted to various salinities. Comp. Biochem. Physiol. 102B: 293-301.

Kültz, D. and Jürss, K. (1991). Acclimation of chloride cells and Na/K-ATPase to energy deficiency in tilapia (*Oreochromis mossambicus*). Zool. Jb. Physiol. 95: 39-59.

Laidley, C.W. and Leatherland, J.F. (1988). Cohort sampling, anaesthesia and stocking-density effects on plasma cortisol, thyroid hormone, metabolite and ion levels in rainbow trout, *salmo gairdneri* Richardson. J. Fish Biol. 33: 73-88.

Laird, L.M. and Oswald, R.L. (1975). A note on the use of benzocaine (Ethyl P-aminobenzoate) as a fish anaesthetic. Fish. Mgmt. 6: 92-94.

Langdon, J.S., Thorpe, J.E. and Roberts, R.J. (1984). Effects of cortisol and ACTH on gill Na^+/K^+-ATPase, SDH and chloride cells in juvenile Atlantic salmon *Salmo salar* L. Comp. Biochem. Physiol. 77A: 9-12.

Larsson, A. and Lewander, K. (1973). Metabolic effects of starvation in the eel, *Anguilla anguilla* L. Comp. Biochem. Physiol. 44: 367-374.

Lasserre, P. (1971). Increase of $(Na^+ + K^+)$-dependent ATPase activity in gills and kidneys of two euryhaline marine teleosts, *Crenimugil labrosus* (Risso, 1826) and *Dicentrarchus labrax* (Linnaeus, 1758), during adaptation to fresh water. Life Sci. 10: 113-119.

Leach, G.J. and Taylor, M.H. (1982). The effects of cortisol treatment on carbohydrate and protein metabolism in *Fundulus heteroclitus*. Gen. Comp. Endocrinol. 48: 76-83.

Leloup-Hatey, J. (1974). Influence de l'adaptation a l'eau de mer sur la fonction interrenalienne de l'Anguille (*Anguilla anguilla* L.) Gen. Comp. Endocrinol. 24: 28-37.

Leloup, J. and De Luze, A. (1985). Environmental effects of temperature and salinity on thyroid function in teleost fishes. *In* "The Endorcine System and the Environment (B.K. Follet, S. Ishii, and A. Chandola, eds.), pp. 23-32. Japan, Sci. Soc. Press, Tokyo/Springer, Berlin.

Lim, A.L.L. and Ip, Y.K. (1989). Effect of fasting on glycogen metabolism and activities of glycolytic and gluconeogenic enzymes in the mudskipper *Boleophthalmus boddaerti*. J. Fish Biol. 34: 349-367.

Loughna, P.T. and Goldspink, G. (1984). The effects of starvation upon protein turnover in red and white myotomal muscle of the rainbow trout *Salmo gairdneri* Richardson. J. Fish Biol. 25: 223-230.

Love, R.M. (1970). The Chemical Biology of Fishes, Vol. I. Academic Press, New York.

Lowe, T.E. and Wells, R.M.G. (1996). Primary and secondary stress responses to line capture in the blue mao mao. J. Fish Biol. 49: 287-300.

Lowry, O.H., Rosebrough, N.A., Farr, A.L. and Randall, R.J. (1951). Protein measurement with the folin phenol reagent. J. Biol. Chem. 193: 264-275.

Machado, C.R., Garofalo, M.A.R., Roselino, J.E.S., Kettelhut, I.C. and Migliorini, R.H. (1988). Effects of starvation, refeeding, and insulin on energy-linked metabolic processes in catfish (*Rhamdia hilarii*) adapted to a carbohydrate-rich diet. Gen. Comp. Endocrinol. 71: 429-437.

Madsen, S.S. (1990a). Cortisol treatment improves the development of hypoosmoregulatory mechanisms in the euryhaline rainbow trout, (*Salmo gairdneri*). Fish Physiol. Biochem. 8: 45-52.

Madsen, S.S. (1990b). Effect of repetitive cortisol and thyroxine injections on chloride cell number and Na^+/K^+-ATPase activity in gills of freshwater acclimated rainbow trout, *Salmo gairdneri*. Comp. Biochem. Physiol. 95A: 171-175.

Madsen, S.S. (1990c). The role of cortisol and growth hormone in seawater adaptation and development of hypoosmoregulatory mechanisms in sea trout parr (*Salmo trutta trutta*). Gen. Comp. Endocrinol. 79: 1-11.

Madsen, S.S. and Bern, H.A. (1992). Antagonism of prolactin and growth hormone : impact on seawater adaptation in two salmonids, *Salmo trutta* and *Oncorhynchus mykiss*. Zool. Sci. 9: 775-784.

Madsen, S.S. and Bern, H.A. (1993). *In-vitro* effects of insulin like growth factor-I on gill Na^+,K^+-ATPase in coho salmon *Oncorhynchus kisutch*. J. Endocrinol. 138: 23-30.

Madsen, S.S., McCormick, S.D., Young, G., Endersen, J.S., Nishioka and Bern, H.A. (1994). Physiology of seawater acclimation in the striped bass, *Morone saxatilis* (Walbaum). Fish Physiol. Biochem. 13: 1-11.

Madsen, S.S. and Naamansen, E.T. (1989). Plasma ionic regulation and gill Na/K-ATPase changes during rapid seawater transfer of yearling rainbow trout (*Salmo gairdneri*): Time course and seasonal variations. J. Fish Biol. 34: 829-840.

Maetz, J. (1969). Observations on the role of the pituitary-interrenal axis in the ion regulation of the eel and other teleosts. Gen. Comp. Endorcrinol. Suppl. 2: 299-316.

Mancera, J.M., Pérez-Figares, J.M. and Fernández, Llebrez, P. (1993). Osmoregulatory response to abrupt salinity changes in the euryhaline gilthead sea bream (*Sparus aurata L.*). Comp. Biochem. Physiol. 95A: 171-175.

Mancera, J.M., Pérez-Figares, J.M. and Fernández-Llebrez, P. (1994). Effect of cortisol on brackish water adaptation in the euryhaline gilthead sea bream (*Sparus aurata L.*). Comp. Biochem. Physiol. 107A: 397-402.

Marc, M.A., Quentel, C., Severe, A., Le Bail, P.Y. and Boeuf (1995). Changes in some endocrinological and non-specific immunological parameters during seawater exposure in the brown trout. J. Fish Biol. 46: 1065-1081.

Mayer, I.,Borg, B. and Plisetskaya, E.M. (1994). Plasma levels of insulin and liver glycogen content in one- and two-year old Atlantic salmon (*Salmo salar L.*) during the period of parr-smolt transformation. Fish Physiol. Biochem. 13: 191-197.

Mayer-Gostan, N. and Naon, R. (1992). Effects of ambient ion concentration on gill ATPases in fresh water eel, *Anguilla anguilla*. Fish Physiol. Biochem. 10: 75-89.

McCormick, S.D., Moyes, C.D. and Ballantyne, J.S. (1989). Influence of salinity on the energetics of gill and kidney of Atlantic salmon (*Salmo salar*). Fish Physiol. Biochem. 6: 243-254.

McCormick, S.D. and Saunders, R.L. (1987). Preparatory physiological adaptations for marine life of salmonids: Osmoregulation, growth and metabolism. Am. Fish. Soc. Symp. 1: 211-229.

McCormick, S.D., Walton, W.D., Duston, J., Nishioka, R.S. and Bern, H.A. (1991). Developmental differences in the responsiveness of gill Na^+, K^+-ATPase to cortisol in salmonids. Gen. Comp. Endocrinol. 8: 308-317.

McKay, L.R. and Gjerde, B. (1985). The effect of salinity on growth of rainbow trout. Aquaculture 49: 325-331.

Mehner, T. and Wieser, W. (1994). Energetics and metabolic correlates of starvation in juvenile perch (*Perca fluviatilis*). J. Fish Biol. 45: 325-333.

Milne, R.S. and Leatherland, J.F. (1980). Studies on the relationship between osmotic or ionic regulation and thyroid gland activity in two salmonid fishes *Salmo gairdneri* Richardson and *Oncorhynchus kisutch* walbaum. J. Fish Biol. 16: 349-360.

Moon, T.W. and Johnston, I.A. (1980). Starvation and the activities of glycolytic and gluconeogenic enzymes in skeletal muscles and liver of the plaice, *Pleuronectes platessa*. J. Comp. Physiol. 136B: 31-81.

Morgan, J.D. and Iwama, G. (1991). Effects of salinity on growth, metabolism, and ion regulation in juvenile rainbow and steelhead trout (*Oncorhynchus mykiss*) and fall chinook salmon (*Oncorhynchus tshawytscha*). Can. J. Fish. Aq. Sc. 48: 2083-2094.

Motais, R., Garcia Romeu, F. and Maetz, J. (1965). Mechanisme de l'euryhalinite etude comparee du flet (euryhalin) et du Serran (stenohalin) an cours du transfert en eau douce. C.R. Acad. Sci. (Paris) 261: 801-804.

Motais, R., Garcia Romeu, F. and Maetz, J. (1966). Exchange diffusion effect and euryhalinity in teleosts. J. Gen. Physiol. 50: 391-422.

Motais, R. Isaia, J., Rankin, J.C. and Maetz, J. (1969). Adaptive changes of the water permeability of the teleotean gill epithelium in relation to external salinity. J. Exp. Biol. 51:529-546.

Müller, R. and Hanke, W. (1974). The effects of adrenocortical hormones on osmomineral regulation and carbohydrate metabolism in the roach, *Leuciscus rutilis* Gen. Comp. Endocrinol. 22: 381.

Nance, J.M., Masoni, A., Sola, F. and Bornancin, M. (1987). The effects of starvation and sexual maturation on sodium transbranchial fluxes following direct transfer from fresh-water to sea-water in rainbow trout (*Salmo gairdneri*). Comp. Biochem. Physiol. 87A: 613-622.

Narasimhan and Sundararaj, B.I. (1971). Effects of stress on carbohydrate metabolism in the teleost *Notopterus notopterus* (Pallas). J. Fish Biol. 3: 441-451.

Nayyar, M. (1992). Changes in the profiles of cortisol and carbohydrates during osmoregulatory adjustments of an air-breathing fish in higher salinities. M. Phil. Dissertation, Deptt. of Zoology, A.M.U. Aligarh (India).

Nichols, D.J. and Weisbart, M. (1985). Cortisol dynamics during seawater adaptation of Atlantic salmon, *Salmo salar*. Amer. J. Physiol. 248: R651-R659.

Nielsen, M.E., Boesgaard, L., Sweeting, R.M., McKeown, B.A. and Rosenkilde, P. (1994). Plasma levels of lactate, potassium, glucose, cortisol, growth hormone and triiodo-L-thyronine in rainbow trout (*Oncorhynchus mykiss*) during exercise at various levels for 24 h. Can. J. Zool. 72: 1643-1647.

Olsen, Y.A., Reitan, L.J. and Roed, K.H. (1993). Gill Na^+, K^+-ATPase activity, plasma cortisol level, and non-specific immune response in Atlantic salmon (*Salmo salar*) during parr-smolt transformation. J. Fish Biol. 43: 559-573.

Ottolenghi, C., Puviani, A.C., Ricci, D., Brighenti, L. and Morsiani, E. (1995). The effect of high temperature on blood glucose level in two teleost fish (*Ictalurus melas* and *Ictalurus punctatus*). Comp. Biochem. Physiol. 111A: 229-235.

Parwez, I. (1982). Studies on the physiology of osmoregulation in a freshwater catfish, *Heteropneustes fossilis* (Bloch). Ph.D. Thesis, University of Delhi, India.

Parwez, I. and Goswami, S.V. (1985). Effects of prolactin, adrenocorticotropin, neurohypophysial peptides, cortisol and androgens on some osmoregulatory parameters of the hypophysectomized catfish, *Heteropneustes fossilis* (Bloch). Gen. Comp. Endocrinol. 58: 51-68.

Parwez, I., Goswami, S.V. and Sundararaj, B.I. (1979). Salinity tolerance of the freshwater catfish, *Heteropneustes fossilis* (Bloch). Indian J. Exp. Biol. 17: 810-811.

Parwez, I., Goswami, S.V. and Sundararaj, B.I. (1984). Effect of hypophysectomy on some osmoregulatory parameters of catfish, *Heteropneustes fossilis* (Bloch). J. Exp. Zool.

229: 375-381.

Parwez, I., Sherwani, F.A. and Goswami, S.V. (1994). Osmoregulation in the stenohaline freshwater catfish, *Heteropneustes fossilis* (Bloch) in deionized water. Fish Physiol. Biochem. 13: 173-181.

Pastoureaud, A. (1991). Influence of starvation at low temperature on utilisation of energy reserves, appetite recovery and growth character in sea bass, *Dicentrarchus labrax*. Aquaculture 99: 167-178.

Patino, R., Schreck, C.B., Blanks, J.L. and Zaugg, W.S. (1986). Effects of rearing conditions on the developmental physiology of smolting coho salmon. Trans. Am. Fish. Soc. 115: 828-837.

Paxton, R., Gist, D.H. and Umminger, B.L. (1984). Serum cortisol levels in thermally-acclimated goldfish (*Carassius auratus*) and killifish (*Fundulus heteroclitus*). Implications in control of hepatic glycogen metabolism. Comp. Biochem. Physiol. 78B: 813-816.

Paxton, R. and Umminger, B.L. (1983). Altered activities of branchial and renal Na^+/K^+ and Mg-ATPases in cold-acclimated golfish (*Carassius auratus*). Comp. Biochem. Physiol. 74B: 503-506.

Penington, R.J. (1961). Biochemistry of Dystrophic muscle. Mitochondrial succinate-tetrazolium reductase and adenosine triphosphatase. Biochem. J. 80: 649-654.

Pickering, A.D. and Pottinger, T.G. (1983). Seasonal and diel changes in plasma cortisol levels of the brown trout, *Salmo trutta L.* Gen. Comp. Endocrinol. 49: 232-239.

Pickering, A.D., Pottinger, T.G. and Christie, P. (1982). Recovery of the brown trout, *Salmo trutta, L.*, from acute handling stress: a time-course study. J. Fish Biol. 20: 229-244.

Pickford, G.E., Pang, P.K.T., Weinstein, E., Torretti, J., Hendler, E. and Epstein, F.H. (1970). The response of the hypophysectomized cyprinodont, *Fundulus heteroclitus*, to replacement therapy with cortisol: Effects on blood serum and sodium potassium activated adenosine triphosphatase in the gills, kidney and intestinal mucosa. Gen. Comp. Endocrinol. 14: 524-534.

Porthé-Nibelle, J. and Lahlou, B. (1975). Effects of corticosteroid hormones and inhibitors of steroids on sodium and water transport by goldfish intestine. Comp. Biochem. Physiol. 50A: 801-805.

Provencher, L., Munro, J. and Dutil, J.D. (1993). Osmotic performance and survival of Atlantic cod *Gadus morhua* at low salinities. Aquaculture 116: 219-231.

Redding, J.M., Schreck, C.B., Birks, E.K. and Eving, R.D. (1984). Cortisol and its effects on plasma thyroid hormone and electrolyte concentrations in fresh water and during seawater acclimation in yearling coho salmon *Oncorhynchus kisutch*. Gen. Comp. Endocrinol. 56: 146-155.

Renaud, J.M. and Moon, T.W. (1980). Starvation and the metabolism of hepatocytes isolated from the American eel, *Anguilla rostrata* LeSueur. J. Comp. Physiol. 135: 127-137.

Richman, N.H., III and Zaugg, W.S. (1987). Effects of cortisol and growth hormone on osmoregulation in pre- and desmoltified coho salmon (*Oncorhynchus kisutch*). Gen. Comp. Endocrinol. 65: 189-198.

Roe, J.H. and Dailey, R.E. (1966). Determination of glycogen with the anthrone reagent. Anal. Biochem. 15: 245-250.

Roche, H., Chaar, K. and Pérés. G. (1989). The effect of a gradual decrease in salinity on the significant constituents of tissue in the sea bass (*Dicentrarchus labrax* Pisces). Comp. Biochem. Physiol. 93A: 785-789.

Roche, H., Jouanneteau, J. and Pérés, G. (1983). Effects of adaptation to different salinities on the lipids of various tissue in sea bass (*Dicentrarchus labrax* pisces). Comp. Biochem. Physiol. 74B: 325-330.

Roche, H. and Pérés, G. (1983). Variations de la composition en lipides tissulaires du Loup d'elevage (Dicentrarchus labrax) au cours des quatre saisons. Etude comparative a deux salinites (36 et 4 p. mille). Vie Marine 5: 35-40.

Rockstein, M. and Herron, P.W. (1951). Colorimetric determination of inorganic phosphate in microgram

quantities. Anal. Chem. 23: 1500-1501.

Rush, S.B. and Umminger, B.L. (1978). Elimination of stress-induced changes in carbohydrate metabolism of goldfish (*Carassius auratus*) by training. Comp. Biochem. Physiol. 60A: 69-73.

Sargent, J.R., Thomson, A.J. and Bornancin, M. (1975). Activities and localization of succinic dehydrogenase and Na^+/K^+-activated adenosine triphosphatase in the gills of fresh water and sea water eels (*Anguilla anguilla*). Comp. Biochem. Physiol. 51B: 75-79.

Saurez, R.K. and Mommsen, T.P. (1987). Gluconeogenesis in teleost fishes. Can. J. Zool. 65: 1869-1882.

Sayer, M.D.J. and Reader, J.P. (1996). Exposure of goldspiny, rock cook and corkwing wrasse to low temperature and low salinity: survival, blood physiology and seasonal variations. J. Fish Biol. 49: 41-63.

Schreck, C.B., Whaley, R.A., Boss, N.L., Naughan, O.E. and Solazzi, N. (1976). Physiological responses of rainbow trout (*Salmo gairdneri*) to electroshock. J. Fish. Res. Bd. Can. 33: 76-84.

Schwarzbaum, P.J., Wieser, W. and Cossin, A.R. (1992). Species-specific responses of membranes and the Na^++K^+ pump to temperature change in the kidney of two species of freshwater fish, roach (*Rutulis rutulis*) and Arctic char (*Salvelinus alpinus*). Physiol. Zool. 65: 17-34.

Schwarzbaum, P.J., Wieser, W. and Niederstatter, H. (1991). Contrasting effects of temperature acclimation on mechanisms of ionic regulation in an eurythermic and stenothermic species of freshwater fish (*Rutulis rutulis* and *Salvelinus alpinus*). Comp. Biochem. Physiol. 98A: 483-489.

Selye, H. (1950). Stress and the general adaptation syndrome. Brit. Med. J. 1: 1383-1392.

Sheridan, M.A. (1989). Alterations in lipid metabolism accompanying smoltification and seawater adaptation of salmonid fish. Aquaculture 82: 191-203.

Sheridan, M.A. and Mommsen, T.P. (1991). Effect of nutritional status on *in vivo* lipid and carbohydrate

metabolism of coho salmon, *Oncorhynchus kisutch*. Gen. Comp. Endocrinol. 81: 473-483.

Sheridan, M.A., Woo, N.Y.S. and Bern, H.A. (1985). Changes in the rate of glucogenesis, glycogenolysis, lipogenesis and lipolysis in selected tissues of the coho salmon (*Oncorhynchus kisutch*) associated with parr-smolt transformation. J. Exp. Zool. 236: 35-44.

Shivakumar, K. and Jayaraman, J. (1984). Salinity adaptation in fish : Effect of thyroxine on mitochondrial status. Archs. Biochem. Biophys. 233: 728-735.

Slicher, A.M., Pickford, G.E. and Pang, P.K.T. (1966). Effects of "training" and of volume and composition of the injection fluid on stress-induced leukopenia in the mummichog. Prog. Fish-Cult. 28: 216-219.

Smith, H.W. (1932). Water regulation and its evolution in fishes. Quart. Rev. Biol. 7: 1-26.

Snedecor, G.W. and Cochran, W.G. (1971). Statistical methods. Iowa State University Press, Ames, Iowa.

So, S.T.C. and Chan, D.K.O. (1985). Metabolic changes during adaptation to seawater in the eel, *Anguilla japonica*: role of hormones. *In* "Current Trends in Comparative Endocrinololy" (B. Lofts and W,N. Holmes, eds.), pp. 922-927. Hong Kong University Press, Hong Kong.

Soengas, J.L., Aldegunde, M. and Andres, M.D. (1995a). Gradual transfer to sea water of rainbow trout : effect on liver carbohydrate metabolism. J. Fish Biol. 47: 466-478.

Soengas, J.L., Barciela, P., Aldegunde, M. and Andres, M.D. (1995b). Gill carbohydrate metabolism of rainbow trout is modified during gradual adaptation to sea water. J. Fish Biol. 46: 845-856.

Soengas, J.L., Barciela, P., Fuentes, J., Otero, J., Andres, M.D. and Aldegunde, M. (1993). The effect of seawater transfer in liver carbohydrate metabolism of domesticated rainbow trout (*Oncorhynchus mykiss*). Comp. Biochem. Physiol. 105B: 337-343.

Sovivo, A., Nyholm, K. and Huhti, M. (1977). Effects of anesthesia with MS222, neutralized MS222 and benzocaine on the blood constituents of rainbow trout, *Salmo*

gairdneri. J. Fish Biol. 10: 91-101.

Specker, J.L. (1980). Hormonal and osmoregulatory aspects of smoltification in coho salmon, *Oncorhynchus kisutch*. Ph.D. Thesis, Oreogon State University, Corvallis.

Spieler, R.E. (1974). Short-term serum cortisol concentrations in goldfish (*Carassius auratus*) subjected to serial sampling and restraint. J. Fish. Res. Bd. Can. 31: 1240-1242.

Spottle, S., Bubucis, P.M. and Anderson, G. (1991). Plasma cortisol response of seawater-adapted mummichogs (*Fundulus heteroclitus*) during deep MS-222 anesthesia. Zool. Biol. 10: 75-79.

Stagg, R.M. and Shuttleworth, T.J. (1982). Na^+, K^+-ATPase ouabain binding and ouabain-sensitive oxygen consumption in gills from *Platichthys flesus* adapted to seawater and freshwater. J. Comp. Physiol. 147: 93-99.

Staurnes, M., Rainuzzo, J.R., Sigholt, T. and Jorgensen, L. (1994). Acclimation of Atlantic cod (*Gadus morhua*) to cold water: Stress response, osmoregulation, gill lipid composition and gill Na-K-ATPase activity. Comp. Biochem. Physiol. 109A: 413-421.

Strange, R.J. and Schreck, C.B. (1978). Anesthetic and handling stress on survival and cortisol concentration in yearling chinook salmon (*Oncorhynchus tshawytscha*). J. Fish Res. Bd. Can. 35: 345-349.

Stuenkel, E.L. and Hillyard, S.D. (1980). Effects of temperature and salinity on gill Na^+-K^+-ATPase activity in the pupfish, *Cyprinodon salinus*. Comp. Biochem. Physiol. 67A: 179-182.

Sun, L.T., Chen, G. and Chang, C. (1994). Characteristics of blood parameters and gill Na^+-K^+-ATPase in chilled comatose tilapia cultured in various salinities. Comp. Biochem. Physiol. 107A: 641-646.

Tagawa, M. and Hirano, T. (1987). Presence of thyroxine in eggs and changes in its content during early development of chum salmon, *Oncorhynchus keta*. Gen. Comp. Endocrinol. 68: 129-135.

Takei, Y. (1993). Role of peptide hormones in fish osmoregulation. *In.* "Fish Endocrinology" (J.C. Rankin and F.B. Jensen, eds.), pp. 136-160. Chapman and Hall, London.

Thomas, P. and Robertson, L. (1991). Plasma cortisol and glucose stress responses of red drum (*Sciaenops ocellatus*) to handling and shallow water stressors and anesthesia with MS-222, quinaldine sulfate and metomidate. Aquaculture 96: 69-86.

Umminger, B.L. (1973). Death induced by injection stress in cold-acclimated goldfish, *Carassius auratus*. Comp. Biochem. Physiol. 45A: 883-887.

Utida, S., Hirano, T., Oide, H., Ando, M., Johnson, D.W. and Bern, H.A. (1972). Hormonal control of the intestine and urinary bladder in teleost osmoregulation. Gen. Comp. Endocrinol., Suppl. 3: 317-327.

Veillette, P.A., Sundell, K. and Specker, J.L. (1995). Cortisol mediates the increase in intestinal fluid absorption in Atlantic salmon during parr-smolt transformation. Gen. Comp. Endocrinol. 97: 250-258.

Vijayan, M.M., Ballantyne, J.S. and Leatherland, J.F. (1991). Cortisol-induced changes in some aspects of the intermediary metabolisms of *Salvelinus fontinalis*. Gen. Comp. Endocrinol. 82: 476-486.

Vijayan, M.M. and Leatherland, J.F. (1990). High stocking density affects cortisol secretion and tissue distribution in brook charr, *Salvelinus fontinalis*. J. Endocrinol. 124: 311-318.

Vijayan, M.M. and Moon, T.W. (1992). Acute handling stress alters hepatic glycogen metabolism in food-deprived rainbow trout (*Oncorhynchus mykiss*). Can. J. Fish. Aquat. Sci. 49: 2260-2266.

Vijayan, M.M. and Moon, T.W. (1994). The stress response and the plasma disappearance of corticosteroid and glucose in a marine teleost, the sea raven. Can. J. Zool. 72: 379-386.

Virtanen, E. (1987). Correlations between energy metabolism, osmotic balance and external smolt indices in smolting young salmon, *Salmo salar L.* Ann. Zool. Fennici, 24: 71-78.

Waring, C.P., Stagg, R.M. and Poxton, M.C. (1992). The effects of handling on flounder (*Platichthys flesus L.*) and Atlantic salmon (*Salmo salar L.*). J. Fish Biol. 41: 131-144.

Wedemeyer, G.A. (1970). Stress of anaesthesia with MS222 and benzocaine in rainbow trout (*Salmo gairdneri*). J. Fish. Res. Bd. Can. 27: 909-914.

Wedemeyer, G.A. (1972). Some physiological consequences of handling stress in juvenile steelhead trout (*Salmo gairdneri*) and coho salmon (*Oncorhynchus kisutch*). J. Fish. Res. Bd. Can. 29: 1780-1783.

Wedemeyer, G.A., Barton, B.A. and McLeay, D.J. (1990). Stress and acclimation. *In* "Methods for Fish Biology", (C.B. Schreck and P.B. Moyle, eds.), pp. 451-489. American Fisheries Society. Bethesda, MD.

Woo, N.Y.S. and Cheung, S.I. (1980). Metabolic effects of starvation in the snakehead, *Ophiocephalus maculatus*. Comp. Biochem. Physiol. 67A: 623-627.

Woo, N.Y.S. and Chung, K.C. (1995). Tolerance of *Pomacanthus imperator* to hypoosmotic salinities : Changes in body composition and hepatic enzyme activities. J. Fish Biol. 47: 70-81.

Woo, N.Y.S. and Fung, A.C.Y. (1981). Studies on biology of the red sea bream, *Chrysophrys major*-II. Salinity adaptation. Comp. Biochem. Physiol. 69: 237-242.

Woo, N.Y.S. and Murat, J.C. (1981). Studies on the biology of red sea bream *Chrysophrys major*. III. Metabolic responses to starvation in different salinities. Mar. Biol. 61: 255-260.

Woo, N.Y.S. and Wu, R.S.S. (1982). Metabolic and osmoregulatory changes in reduced salinities in the red grouper, *Epinephelus akaara* (Temminck and Schlegel), and the black sea bream, *Mylio macrocephalus* (Balsilewsky). J. Exp. Mar. Biol. Eco. 65: 139-161.

Young, G. (1986). Cortisol secretion *in vitro* by the interrenal of coho salmon (*Oncorhynchus kisutch*) during smoltification: relationship with plasma thyroxine and plasma cortisol. Gen. Comp. Endocrinol. 63: 191-200.

Zwingelstein, G., Portoukalian, J., Rebel, G. and Brichon, G. (1980). Gill sulfolipid synthesis and seawater adaptation in euryhaline fish *Anguilla anguilla*. Comp. Biochem. Physiol. 65B: 555-558.